The Functional Significance of Fruit Exocarp on Host Selection and Oviposition by Queensland Fruit Fly, *Bactrocera tryoni* (Froggatt) (Tephritidae: Diptera)

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Abstract

In this thesis I investigated the effect of host peel toughness on ovipositor wear and host range in the polyphagous Queensland fruit fly, *Bactrocera tryoni* (Froggatt). Specifically, I inquired whether ovipositing *B. tryoni* would accept or reject hosts based upon the penetrability of host fruit exocarp and the presence of peel punctures. In a related line of inquiry, I also examined whether the aculei (= the cuticular ovipositor tip) of female *B. tryoni* would experience different levels of abrasive wear after prolonged exposure to soft and hard-peeled hosts. I started this thesis with two key assumptions based on the existing literature: (i) that the aculei of fruit flies exposed to hosts with hard peel would experience wear; and (ii) that ovipositing *B. tryoni* females would display a preference for soft peeled hosts over hard-peeled hosts. Although presumed connections between host peel toughness, ovipositor wear and host range within tephritid fruit flies are raised regularly in the tephritid literature, a comprehensive investigation into these issues had not been performed prior to my study.

To investigate aculei wear, caged populations of *B. tryoni* were exposed to a variety of natural and artificial hosts for seven weeks. Aculei samples were taken weekly and examined for signs of abrasive wear, which revealed that while aculeus wear in *B. tryoni* as a product of oviposition substrate or time did occur, it was very limited and restricted to my artificial host substrates: no significant wear was observed in real fruit of varying peel toughness. A general lack of wear was confirmed with samples of wild flies from the field, where evidence of ovipositor wear was also minimal. Laboratory-based choice and no-choice experiments showed that increased peel toughness or peel surface properties (waxiness and roughness) did not act as

deterrents to ovipositing *B. tryoni* females. Passionfruit, scored as a high quality host for larval development, was selected over hosts of less quality for larvae, despite those fruit having softer peels. However, the extremely tough peel of passionfruit did physically stop *B. tryoni* oviposition in most ovipositor probing attempts. These results reject a case of *B. tryoni* selecting hosts based on physical ease of oviposition, but are best explained by the preference-performance hypothesis of herbivore host selection.

The observations of unworn *B. tryoni* aculei, coupled with the fly's behavioural selection of hard-peeled hosts for oviposition, led me to explore mechanisms that might explain the low levels of ovipositor wear observed. Comparative X-ray microanalysis of aculei belonging to *B. tryoni,* olive fruit fly (*Bactrocera oleae* Gmelin) and Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) revealed that transition metals, known to act as cuticular hardening agents in other insects, were not present. The second wear reduction mechanism I investigated was the use of damaged or soft sections of host peel: prior studies of *B. tryoni* have reported that females will often reuse ovipunctures made by conspecific females. Although flies in field cage trials did not demonstrate a significant prealighting preference for hosts with damaged peel, when such hosts were alighted on subsequent host handling time was much shorter. This suggests that the previously recorded preference by *B. tryoni* for fruit with damaged peel (a behaviour which has also been recorded for other tephritids) is a mechanism to optimise host handling time, not a mechanism to overcome peel physical constraints. In the same trial, I also confirmed earlier reports that maggot infested hosts were not oviposited into, and indeed they were very rarely alighted upon. Chemical analysis of these hosts revealed elevated levels of the bacterially

produced chemical acetoin, which was not recorded in the other host treatment groups and which may be a mechanism by which infested fruit are detected by conspecific females.

My investigations into the mechanistic interactions between *B. tryoni* and host fruit have identified key processes driving host-use patterns. Specifically, I conclude that host-use decisions are not made on peel attributes, and that everything except fruit of extreme peel toughness can be utilised as a host with little sign that host peel properties caused selective oviposition or ovipositor wear. Rather, the preferential use of fruit with damaged peel has significant time saving benefits to female flies, which may (although this was not assessed) have flow-on benefits in terms of minimising predator induced mortality or optimising oviposition behaviour.

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Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Date: August 2014

Signed: QUT Verified Signature

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Chapter 1: General Introduction and Literature Review

1.1 Introduction

The phytophagous insects of the family Tephritidae (Diptera), or the true fruit flies, are an extremely diverse group, composed of more than 5000 species in 481 genera (Norrbom *et al*., 1999). All tephritids are phytophagous, but not all (despite their common name) are frugivorous. For example, the sub-family Tephritinae almost exclusively use the developing seed-heads of daisies as the substrate for larval development (Mazzon *et al*., 2008). However, in this thesis, the focus is on the frugivorous tephritids which includes the four major genera of pest fruit flies: *Ceratitis* MacLeay, *Bactrocera* Macquart, *Anastrepha* Schiner and *Rhagoletis* Loew (Malacrida *et al*., 2007). The Tephritidae are distributed in temperate, tropical and sub-tropical regions of the world, although individual genera have more restricted distributions. For example *Ceratitis* is an Afrotropical genus, *Bactrocera* is mainly confined to the Oriental and Australasian regions, *Anastrepha* to South and Central America and the West Indies, while *Rhagoletis* is found in North and Central America, Europe and temperate Asia (Bateman, 1972; Fletcher, 1987; Headrick & Goeden, 1998; Carey, 2011).

Frugivorous female fruit flies use fruit as a substrate into which to lay their eggs and the larval stage subsequently develop there. A specialised structure known as the ovipositor is used to penetrate the peel (i.e. exocarp) of the fruit, allowing the female to deposit her eggs within the fruit flesh (i.e. pericarp) (Sumrnadee *et al*., 2011). Oviposition behaviour is a very important element of tephritid behaviour because it directly influences the potential survival and development of larvae; from an applied perspective it is also the behavioural aspect which makes fruit flies crop pests (Nguyen *et al*., 2007). Economic losses resulting from direct fruit fly damage and

associated pest control efforts in Australia alone are considerable, with costs estimated at $125 million per annum (Yonow & Sutherst, 1998; Clarke *et al*., 2011). Such losses are relatively mild when compared to international examples. Approximately $800 million per annum is lost due to olive fruit fly (*Bactrocera oleae* (Gmelin)) in the Mediterranean basin (Daane & Johnson, 2010). Considerable indirect losses also result when access to foreign markets is curtailed because of fruit fly threat (De Meyer *et al*., 2010; Khamis *et al*., 2012). Immature fruit fly stages (*i.e.* the eggs and larvae) can be transported in fruit and so pose an unacceptable quarantine risk unless treated (Drew, 2001a), resulting in some governments putting into place strict quarantine measures designed to curtail the spread of flies into unaffected areas (Yonow & Sutherst, 1998; De Meyer *et al*., 2010; Rengifo *et al*., 2011).

The economic damage arising from tephritid oviposition has stimulated the interest of researchers, typically directed towards economically significant pest species (Papaj *et al*., 1989ab; Jang & Light, 1991; Cornelius *et al*., 2000; Joachim-Bravo *et al*., 2001ab; Rattanapun *et al*., 2009). Aspects of fruit fly ecology such as host plant identification and selection, oviposition behaviour, mating behaviour, biology and superparasitism have been studied in depth (Christenson & Foote, 1960; Messina *et al*., 1991; Prokopy *et al*., 1999; Dukas *et al*., 2001; Balagawi *et al*., 2005). Nevertheless, there are still important omissions within the established scientific literature and much assumed knowledge. With respect to this thesis, little detailed knowledge is available regarding the impact of fruit peel qualities and aculeus morphology on fruit fly oviposition behaviour. It is assumed that a link between insect ovipositor morphology, oviposition behaviour, peel toughness and host-use exists (Groman & Pellmyr, 2000; Sayar *et al*.,

2009), but detailed studies are very limited (those available are reviewed later in this chapter).

In this thesis I explore in detail the relationship between ovipositor morphology, oviposition behaviour and host-use in fruit flies, focusing particularly on the polyphagous *Bactrocera tryoni* (Froggatt) (Tephritidae: Dacinae). In preparation for the experimental chapters the following literature review examines three broad areas of interest which are essential for understanding the theoretical and experimental background to my thesis: (i) the biology, life cycle and ecology of tephritid fruit flies, with an emphasis on the principal study organism; (ii) a section on herbivore host range, including an introduction to a selection of relevant theoretical host range models; and (iii) an examination of fruit fly oviposition behaviour, ovipositor morphology and the negative consequences of increased ovipositor wear caused by impenetrable host peel. This chapter is concluded by a section in which specific research questions are outlined and the flow of the experimental chapters is presented.

1.2 Introduction to Dacine Tephritids, with a Focus on the Queensland Fruit Fly

The Dacini is the largest tribe within the sub-family Dacinae of the Tephritidae, and consists primarily of two major genera, *Bactrocera* Macquart and *Dacus* Fabricius (Drew & Romig, 2007; Krosch *et al*. 2012). Key ecological characteristics of dacine fruit flies include high mobility, high fecundity and relatively long adult life spans (Fletcher, 1989). Dacine fruit flies have a wide geographical distribution and may be found in tropical, subtropical and temperate regions of the world (Raghu, 2002; Balagawi, 2006; Clarke *et al*., 2011). At the habitat level, species of Dacini have been

found in habitats ranging from open sclerophyll forests, rainforests and heavily disturbed suburban areas (Raghu *et al*., 2000). Polyphagous tephritids such as *B. tryoni* are strong fliers, and may travel upwards of 90 km during the post-teneral period prior to host seeking and mating or when local hosts become unavailable (Bateman, 1972; Fletcher, 1987; MacFarlane *et al*., 1987). However, more recent results (e.g. Meats and Edgerton 2008) suggest that most *B. tryoni* will not disperse farther than one kilometre.

The great majority of dacine fruit flies are frugivorous in the larval stage of their life-cycle. In contrast to the larval stage, adult fruit flies are free-living in the environment. A wide variety of food sources are used by adult flies, including plant glandular secretions, nectar, plant sap, rotting fruit, bird dung and decaying insects (Christenson & Foote, 1960). Fruit flies are classified as minor or major agricultural pests when larval hosts include commercially cultivated crops (Salazar *et al*., 2002; Aluja & Mangan, 2008).

1.2.1 The Life Cycle and Ecology of Dacine Fruit Flies with a focus on *Bactrocera tryoni*

Nearly all dacine fruit flies possess a similar life cycle, although there are differences in life history traits between species such as the number of generations per year, number of eggs produced per female and host range (Bateman, 1972; Muthuthantri, 2008). Gravid female fruit flies lay either a single egg or clutch of eggs into the flesh of ripening fruit. The larvae hatch from the eggs after approximately 42 hours (at 25º C) and feed inside the plant, causing direct fruit damage and inducing bacterial decay (Bateman, 1972; Clarke *et al.,* 2011). The life cycle of dacine fruit flies can be

divided into five stages: egg; larva; pupa; teneral adult; and reproductive adult (Yonow *et al*., 2004).

The life cycle of tephritid fruit flies is regulated by environmental conditions including light, temperature and moisture (Florec *et al*., 2013). Within warm areas *B. tryoni* adults will breed throughout the year, although breeding will cease in winter if mean temperatures fall below the thresholds required for ovarian maturation (13.5^{o}C) and mating (16^{o}C) (O'Loughlin *et al*., 1984; Muthuthantri *et al*., 2010). During such conditions flies will retreat to sheltered refuge sites until conditions become more favourable (Fletcher, 1979). Egg laying occurs between the temperatures of 18.5^{o}C and 34^{o}C, and is often depressed during the hottest parts of the day and periods of heavy rainfall (Bateman, 1972; Yonow *et al*., 2004). Gravid *B. tryoni* females will lay their eggs in clutches of 3-6 under the peel of the host fruit. Eggs take two days to hatch (Fletcher, 1969; Fitt, 1990).

Dacini larval development times are dependent upon a number of factors including fruit species, maturity, temperature, larval density and moisture, and vary from 7 to 10 days for different species of tephritid fruit flies (Bateman, 1979; Seo *et al*., 1983; Averill & Prokopy, 1987; Krainacker *et al*., 1987; Meats, 1989; Reynolds *et al*., 2010; Quesada-Moraga *et al*., 2012). Upon hatching the larvae will begin feeding upon the flesh of the fruit, passing through three instars (Meyers, 1952; Christenson & Foote, 1960). The larvae of most species ingest liquid food, making use of "fruit fly-type" bacteria to rot down the fruit tissue and produce a bacterial soup which may provide essential nutrients or may be ingested directly as food (Drew & Lloyd, 1991; Drew & Yuval, 2001). After 7 to 10 days, *B. tryoni* prepupae use their mouth-hooks to cut

open the skin of the host fruit, before burrowing and pupating in the top layer (2-3 cm) of the soil for approximately 12 days at 25^{o}C before emergence (Christenson & Foote, 1960; Bateman, 1967; Bateman, 1972; Meats, 1981; Fletcher, 1987). Rapid larval growth and a short pupal period are hypothesised to be life history strategies which minimise contact with potential predators (Fletcher, 1989). The emergent adults are sexually immature, and the time to sexual maturation varies greatly between species, with *B. tryoni* reaching sexual maturity approximately 8-10 days after emergence (Christenson & Foote, 1960; Meats, 1981; Fletcher, 1987).

The ability of teneral adults to mature, mate and lay eggs depends on access to various resources within the environment, as well as appropriate environmental conditions. The dietary requirements needed by adult tephritids to survive and reproduce include amino acids, vitamins, minerals, carbohydrates and water; these are obtained from a wide variety of sources within the environment (Fletcher, 1987; Raghu, 2002). The majority of these nutrients are acquired by foraging during the adult phase, although some may be carried over from the larval stage, synthesised *de novo* by flies after the ingestion of necessary precursors, or supplied by symbiotic organisms (Daser & Brandl, 1992; Drew & Yuval, 2001). When sexual maturity is achieved, adult flies forage for mates using a variety of courtship behaviours involving visual, auditory and chemical cues (Sivinski *et al*., 2001). Although males can mate frequently, females can become sexually unreceptive for several weeks after mating (Fletcher, 1987), although they may also remate quite quickly (Kumaran *et al*., 2013). After mating, females will switch from mating behaviour to host location and oviposition behaviour. Gravid females may lay between 80-100 eggs per week, or an average of

11-14 eggs per day, over a lifespan of several months (Bateman, 1979; Meats, 1981; Dominiak *et al*., 2008).

1.3 Host Range in Dacine Fruit Flies

"Host-use" in phytophagous insects is generally used in reference to the plant species consumed or oviposited upon; - whereas the selection of a particular host plant over another under the same environmental conditions is referred to as "host preference" (Singer, 1983). The over-arching term 'host preference' consists of three linked components; i) the attractiveness of a host to a pre-alighting female; ii) whether or not the insect accepts the host after alighting upon its surface; and iii) the ability of the host to sustain larvae (Cunningham *et al*., 1998; Robacker & Fraser, 2002).

Phytophagous insects, including dacine fruit flies, use host plants (or specific parts of them) for many different activities including sheltering, feeding, mating, oviposition and larval development (Thorsteinson, 1953; Balagawi, 2006). Drew & Lloyd (1987) have claimed that the larval host plant is the "centre of activity" for fruit flies, and that all activities performed by the flies are based around it. Although the importance of the host plant cannot be dismissed, it is inappropriate to suggest that it is the sole focus of fruit fly activity (Raghu *et al*., 2002).

Plant feeding insects are typically classified into three broad groups according to the size of the host range. Monophagous (= specialist) insects feed on only one plant species, oligophagous insects on a narrow range of related plant species, while polyphagous (= generalist) insects feed on a wide range of unrelated plant species,

typically crossing plant families (Prokopy & Owens, 1983; Bernays & Chapman, 1994). There is little consensus when it comes to the definition of these terms (Kelley & Farrell, 1998), but in this thesis monophagy will conform to Bernays & Chapman's (1994) description as feeding on a single plant species. I do note, however, that others have defined it differently, from feeding on several plant species within a genus (Stark, 1982), or feeding on plant species within a plant family (Muller, 1996).

While polyphagous insects are regarded as opportunists and will use hosts that are rare, hard to find, or unpredictable in the environment, monophagous insects are thought to use abundant and easily found plants (Jaenike, 1990). The advantages of polyphagy over monophagy are regarded as considerable (Bernays & Graham, 1988; Walter, 2003), as unlike monophages the survival of a polyphagous herbivore is not dependent upon a single species of plant. While polyphagous insects are seen as somewhat inefficient at using multiple resources in comparison to a specialist, they are also considered to be more flexible in the face of external conditions or variable host quality (Michaud, 1990; Walter, 2003). Monophagous insects may be able to do very well on an abundant single plant species, but if that host species becomes unavailable the specialist is doomed. The polyphagous insect, alternatively, may do only moderately well on a range of plant species, but the chances of all those plant species being unavailable is very low. Polyphagy is therefore seen as a "risk spreading" life-history strategy (Michaud, 1990; Prokopy & Owens, 1983).

Despite the perceived advantages of polyphagy, specialisation is the common rule among phytophagous insects, including fruit flies (Bernays & Chapman, 1994; Drew, 2004; Rasmann & Agrawal, 2011). However, polyphagy has been noted to occur far

more frequently in the predominantly rainforest endemic genus *Bactrocera*, where approximately 40% of fruit fly species are recorded as using hosts from across two or more plant families (Drew, 2004).

1.3.1 Host Range Records and the Differences between Fundamental versus Realised Host Range

The host range size of an insect is typically assessed by published lists of the host plants associated with the insect: examples of host-use records for Dacini fruit flies include those produced by Hancock *et al*. (2000) and Allwood *et al*. (1999). However, such lists rarely consider the issue of host preference, for example Hancock *et al*. (2000) note (for some hosts only) that they are 'major' or 'minor' hosts, but provide no further information on how this categorization was made, or even what it really means. For polyphagous fruit flies, there are noticeable inconsistencies between the long lists of hosts provided in published lists and observations performed during host preference tests, which demonstrate that generalist fruit flies do not treat hosts indiscriminately (Clarke *et al*., 2005; Rwomushana *et al*., 2008). For example, a detailed ecological analysis of the host records complied by Allwood *et al*. (1999) showed that *B. latifrons* should be considered narrowly oligophagous on *Solanaceae* spp, whereas an uncritical assessment of the data in Allwood *et al.* (1999) would lead a reader to classify it as polyphagous (Clarke *et al*., 2001)

A further complication regarding the issue of host range is the critical distinction between fundamental (i.e. physiological) host range and realised (i.e. actual) host range. Realised host range refers to the number of plant species used in the field and is a subset of fundamental host range, which refers to the ability of a herbivorous

insect to successfully complete their development using a particular host (Blossey, 2007). Laboratory tests assessing the fundamental host range of an insect often indicate broad host ranges, which is attributed to the controlled conditions of the laboratory and manipulation of experimental variables (Morehead & Feener Jr, 2000). In contrast, the realised host range of the insect under field conditions are typically narrow, as behavioural, physical, sensory and ecological constraints restrict the insect from using all potential hosts (Balciunas *et al*., 1996; Van Klinken & Heard, 2000). Consequently, it is vital when exploring the issue of host range that it is done from a perspective that takes into account the biological and ecological factors that the fruit fly will face in the field.

The short section above gives a very brief idea of the complexities which can be involved in studying herbivore host range. Given this complexity, herbivore ecologists devote a considerable proportion of their work to developing the theoretical basis for understanding and explaining host range. As host range is a key topic of this thesis, the following section examines some of the key theories.

1.3.2 Selected Host Range Models

Numerous hypotheses and models have been proposed to explain the observed host ranges of phytophagous insects, including the chemical coevolution hypothesis (Jermy, 1984; Bernays & Graham, 1988), plant apparency and chemical defence (Feeny, 1976), time-limitation hypothesis (Levins & MacArthur, 1969; Courtney, 1982; Ward, 1992; Larsson & Ekbom, 1995; Mayhew, 1997), hierarchy threshold hypothesis (Mangel, 1987; Courtney *et al*., 1989), density-dependant models (Rausher, 1984; Jaenike, 1990; Mayhew, 1997) and optimality theory (Jaenike 1978,

1990; Papaj, 1994). A selection of these hypotheses/models is outlined below according to their relevance in interpreting the different experimental results of this thesis.

1.3.2.1 *Optimality Theory (= Preference-Performance Hypothesis)*

Optimality models are prominent in theoretical and empirical studies examining the host preference patterns of phytophagous insects and include the widely used host preference-performance model of host selection (Jaenike 1978, 1990; Papaj, 1994; Scheirs *et al*., 2004; Heisswolf *et al*., 2005; Clark *et al*., 2011). The core assumption of the preference-performance model is that insects will preferentially oviposit on plants according to their suitability for offspring development and survival (Thompson, 1988bc; Mayhew, 1997; Balagawi *et al*., 2013; Clotuche *et al.*, 2013). This theory relates in particular to insects whose larvae have little or no ability to shift to a different host and are dependent upon the mother's choice of host (Clark *et al*., 2011).

Although positive correlations between host preference and larval performance have been recorded (Heisswolf *et al*., 2005; Staley *et al*., 2009; Clark *et al*., 2011), there are a surprisingly large number of poor correlations between oviposition preference and host quality (Mayhew, 1997; Santos *et al*., 2008; Gripenberg *et al*., 2010; Refsnider & Janzen, 2010; Gillespie & Wratten, 2011). A range of different explanations for such 'bad motherhood' decisions have been put forward. Poor quality larval hosts may be accepted by the mother in order to enhance her individual fitness (i.e. longevity and egg production) at the expense of offspring fitness (Mayhew, 2001; Scheirs & De Bruyn, 2002; Uesugi, 2009). In contrast to such 'selfish' motivations, an ovipositing

female fruit fly may select unripe and seemingly poor hosts in order to prevent larvae being consumed along with their host by frugivorous predators and to provide emergent daughters sufficient time to reach maturity and use newly ripened hosts in the same fruiting season (Grewal & Kapor, 1986; Mangel *et al*., 1994; Purcell *et al*., 1994; Yuval & Hendrichs, 2000; Diaz-Fleischer & Aluja, 2003b). Consequently, such 'poor' host decisions may actually improve the chances of survival and increase the total number of offspring produced. Additional traits associated with preferred hosts such as leaf wax load (*i.e.* glossiness) might also lead the insect to use an unfavourable host (Karungi *et al*., 2010).

1.3.2.2 Hierarchical threshold and time limitation hypotheses

While the hierarchy threshold and time limitation hypotheses are often discussed separately, the broad similarities between the two has led me to regard (at least for the purposes of this review) the time-limitation hypothesis as a subset of the hierarchical threshold hypothesis. Both models emphasise the importance of time and egg limitation as factors influencing the host range of herbivores, with host selection the net result of the interaction between host traits and the internal state of the insect, mediated by the general motivation to oviposit (Janz, 2003a; Balagawi, 2005). Hosts are ranked by the insect in an evolutionarily-fixed hierarchy determined by host-specific factors (*i.e.* plant chemistry, host abundance and host predictability), whereas host acceptability is determined by a threshold value partly governed by the insect's internal status (*i.e.* age, egg load) which may change throughout its lifespan (Minkenberg *et al*., 1992; Sadeghi & Gilbert, 2000; Janz, 2003a; Balagawi, 2005; Bird & Kruger, 2006; Almohamad *et al*., 2009). The acceptance of a particular host is determined on whether the stimulus of the host exceeds the motivational threshold of

the insect (Sadeghi & Gilbert, 2000). An assumption of these theories is that although acceptance thresholds may vary, insects will not become specialised on low-ranking hosts and that insects which accept a lower ranking host will also accept all hosts above that host in the rank order (Courtney *et al*., 1989; Prokopy *et al*., 1994).

Both the hierarchical threshold and time limitation models predict that females may come under pressure to accept less suitable hosts when more preferred hosts are rare or absent, or if the lifespan of the herbivore is limited (Levins & MacArthur, 1969; Courtney *et al*., 1989; West & Cunningham, 2002; Stastny *et al*., 2006; Elkin & Marshall, 2007; Rosenheim *et al*., 2008; Refsnider & Janzen, 2010; Berger *et al*., 2012). Female insects with a surplus of eggs may also be less discriminating than egg-limited females (Berger *et al*., 2012). Changes in host acceptability linked to increased age and egg load have been observed among ovipositing drosophilid and tephritid fruit flies. The acceptability threshold of individual *Drosophila busckii* decreased in response to increased egg loads, with the fly making use of lower ranked hosts (Courtney *et al*., 1989; Minkenberg *et al*., 1992). Increasing age in *B. tryoni* caused ovipositing females to be less selective of potential host plants and this change in behaviour was associated with an increase in the egg load of the females (Fitt, 1986b). In contrast, monophagous fruit fly species did not display the same decrease in discrimination (Fitt, 1986b).

Factors that act to reduce the acceptability threshold of the ovipositing insect and increase the acceptance of lower ranked hosts may result in polyphagy, if the loss in average fitness from accepting lower quality hosts is less than the fitness lost from spending time searching for higher quality hosts (Singer, 1971; Courtney & Forsberg,

1988; Elkin & Marshall, 2007). Alternatively, the higher acceptability threshold in less fecund females will result in monophagy due to only highly ranked hosts being accepted in order to maximise the fitness of individual eggs (Mayhew, 1997; Berger *et al*., 2012). A female which persistently rejects suboptimal hosts may lose the chance to deposit eggs before the end of her reproductive cycle, while the chances of her own death due to factors such as predation and harsh weather are simultaneously increased (Stastny *et al*., 2006; Gibbs & Van Dyck, 2009).

1.3.2.3 Neural limitation hypothesis

The neural limitation hypothesis states that generalist insects are at greater risk of making poor host choices than specialists, due to neural constraints on effective information processing within sensory-rich environments (Janz, 2003b; Janz *et al*., 2005; Aluja & Mangan, 2008; Gripenberg *et al*., 2010). 'Poor' decision making refers to the inability to distinguish between high and low quality hosts and a longer time to make decisions (Janz & Nylin, 1997; Janz, 2003b). The inherently constrained processing ability of the insect central nervous system (CNS) imposes time and accuracy costs upon generalists when they are forced to divide limited cognitive resources between the wide range of sensory cues offered by different hosts (Bernays & Wcislo, 1994; Bernays, 2001; Egan & Funk, 2006; Bird & Kruger, 2006; Castells & Berenbaum, 2008; Clark *et al*., 2011; Costa *et al*., 2011). In contrast, specialists have been recorded as making quicker and more accurate host selection decisions (Bernays & Funk, 1999; Janz, 2003b; Egan & Funk, 2006). Specialists avoid time and accuracy costs by focusing their attention upon a few high-contrast cues that stand out from non-host cues, allowing for fast and accurate decisions to be made (Janz *et al*., 2005; Egan & Funk *et al*., 2006; Castells & Berenbaum, 2008). Increased decision-

making time may leave generalists vulnerable to predation, acting as a selective disadvantage against the evolution of broad host ranges (Bernays & Funk, 1999).

1.4 Tephritid Oviposition Behaviour, with a Focus on Host Peel Properties

The previous section examined several theoretical models for explaining host range in phytophagous insects. Of equal importance in understanding host range is an understanding of the mechanical processes and interactions between the insect and its host which form, collectively, the act of oviposition. The current consensus among researchers is that tephritid oviposition behaviour is a dynamic or 'plastic' event, in which behaviour adapts to the interplay between highly variable environmental and physiological cues (Jaenike, 1990; Bernays & Chapman, 1994; Papaj, 2000; Pinero *et al*., 2006; Aluja & Mangan, 2008; Ansari *et al*., 2012; Migani *et al*., 2013). Ovipositing fruit flies will spend time selecting the appropriate host to deposit their eggs in, as a host of high quality offers improved chances of successful larval development (Drew & Romig, 2001; Genc & Nation, 2008; Sharma & Amritphale, 2008). A broad range of host olfactory, visual and contact cues are used ovipositing females to locate and assess the suitability of potential hosts (Pinero *et al*., 2006; Balagawi *et al*., 2005; Sharma & Amritphale, 2008; Rattanapun *et al*., 2009; Brevault & Quilici, 2009; Liu *et al*., 2011; Quilici & Rousse, 2012). Visual cues are believed to be more important at longer ranges, whereas olfactory and tactile cues have greater priority at medium or short distances (Yuval & Hendrichs, 2000; Randlkofer *et al*., 2010)

1.4.1 Tephritid Ovipositor and Fruit Peel

The act of depositing eggs in a host is accomplished through use of the ovipositor, a structure which in fruit flies consists of three highly modified abdominal components: (i) a tubular or conical oviscape; (ii) an elongate, membranous eversible membrane; and (iii) a needle-like or blade-like aculeus (Fletcher, 1987; Diaz-Fleischer *et al*., 2001). The term ovipositor is typically used as a common name for the aculeus only, as this is the segment which penetrates the skin of the host fruit (Quicke *et al*., 1998; Collier & Van Steenwyk, 2003; Dweck *et al*., 2008). The length of the aculeus varies greatly amongst dacine fruit flies, from 1 mm to more than 2 mm (Iwaizumi *et al*., 1997; Mahmood, 2004).

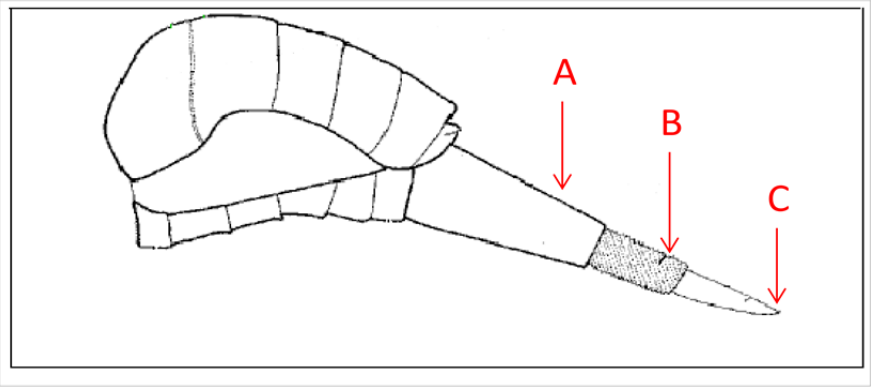


Figure 1.1 Diagram representing the abdomen of a tephritid fruit fly (lateral view). A = oviscape; B = eversible membrane; C = aculeus

Figure 1.2 Light microscope image of an entire *B. tryoni* aculeus

The ability of fruit peel to resist oviposition has been identified as an important factor to consider in understanding tephritid oviposition behaviour (Diaz-Fleischer & Aluja, 2003b; Rouquette & Davis, 2003; Aluja *et al*, 2004; Balagawi *et al*., 2005; Okolle & Ntonifor, 2005; Rattanapun *et al*., 2009, 2010). Tephritids often have difficulty when attempting to penetrate the skin of host fruit (Pritchard, 1969; Messina & Jones, 1990; Jones & Kim, 1994) and several studies have demonstrated that a tough peel will limit the ability of ovipositing females to penetrate the peel and lay their eggs (Messina *et al*., 1991; Balagawi *et al*., 2005; Dhillon *et al*., 2005). In contrast, a positive relationship between increasing oviposition preference and increasing peel penetrability has been identified by several authors (Balagawi *et al*., 2005; Sharma & Amritphale, 2008). Host peel criteria that may positively influence the selection of an oviposition site include soft-skinned areas and rough surfaces; crevices and broken fruit surfaces; and existing oviposition holes made by conspecifics (Bateman, 1972; Balagawi *et al*., 2005; Sidney *et al*., 2008).

Although extremely tough peel has traditionally been viewed as a deterrent to fruit fly oviposition, there is evidence that an increase in peel toughness is not automatically associated with a reduction in oviposition (Pritchard, 1969; Papachristos & Papadopoulos, 2009). Firm host peel may be viewed by ovipositing fruit flies as a favourable trait and used as an indirect measure of host quality, because of the close association between fruit firmness and the maturity of the host (Greany *et al*., 1985; Messina & Jones, 1990; Diaz-Fleischer & Aluja, 2003b).

1.4.2 Cuticular Wear within the Tephritid Ovipositor

All insects (and other arthropods) are characterised by possessing an external cuticle, a composite material that consist of highly crystalline chitin nanofibers embedded in a matrix protein, polyphanols, lipids and water (Rasch *et al*., 2003; Vincent & Wegst, 2004). Cuticle serves a number of vital functions, including structural support and protection from the external environment (Vincent & Wegst, 2004), but the thickness, stiffness, strength and elasticity of insect cuticles can vary greatly depending on the functional role of the specific part of the insect (Andersen, 2010; 2011). Please note that the terms 'hardness' and 'stiffness,' are often used interchangeably and this may lead to confusion when understanding cuticle. Stiffness is a measure of resistance by an elastic body to recoverable deformation, whereas hardness is the characteristic of a solid material expressing its resistance to permanent deformation (Hillerton *et al*., 1982).

Insect 'tools', such as mandibles, claws and ovipositors, that experience significant interaction with high friction materials are generally characterised by possessing cuticle which is hard, elastically stiff, tough and resistant to abrasion (Schofield, 2005; Schoberl & Jager, 2006; Cribb *et al*., 2010). The hardening of cuticle is accomplished primarily through the cross-linking of protein molecules, a process known as tanning or sclerotization, although this does not stop wear in such cuticular structures (Chapman, 1957; 1964; Raupp, 1985; Wallin, 1988; Rasch *et al*., 2003; Roitberg *et al*., 2005; Schofield *et al*., 2011). The addition of transition metals such as zinc to the cuticular matrix has also been linked to increased cuticle hardness (Hillerton & Vincent, 1982; Quicke *et al*., 1998; Schofield *et al*., 2003; Vincent & Wegst, 2004). Wear in cuticular structures is thought to be detrimental to insects, for

example heavily worn mandibles among species of phytophagous ants and grasshoppers have contributed to reduced feeding efficiency (Chapman, 1957; 1964; Raupp, 1985; Wallin, 1988; Roitberg *et al*., 2005; Tammaru & Javois, 2005; Schofield *et al*., 2011).

Only a single study has examined the aculei of tephritid fruit flies for signs of wear (Jones & Kim, 1994). This study identified heavily abraded aculei (i.e. heavily blunted in comparison to newly emerged flies) from four species of tephritid fruit flies; *Rhagoletis pomonella* (Walsh), *R. mendax* (Curran), *Ceratitis capitata* and *Bactrocera oleae*. The aculei of field-collected *C. capitata* were especially worn when compared against laboratory specimens and newly emerged females (Fig. 1.3). This led Jones and Kim to speculate that older flies with worn aculei and decreased vigour would encounter increased difficulty when attempting to penetrate host peel, but of this they presented no actual data. The results and speculations of Jones and Kim (1994) have been widely quoted by other authors (e.g. Okolle & Ntonifor, 2005; Tammaru & Javois, 2005; Genc & Nation, 2008; Rattanapun *et al*., 2009), although no experimental or observational comparisons have ever been made between the oviposition behaviours and host preference patterns of fruit flies with worn or unworn aculei. Consequently, the impact of physical wear upon oviposition and host selection behaviours in fruit flies is still open to question, but the general consensus of the fruit fly literature is that a worn aculeus may lead to host range restrictions within tephritid fruit flies, and that mechanisms might have evolved in order to minimise or avoid wear (Diaz-Fleischer *et al*., 2001).

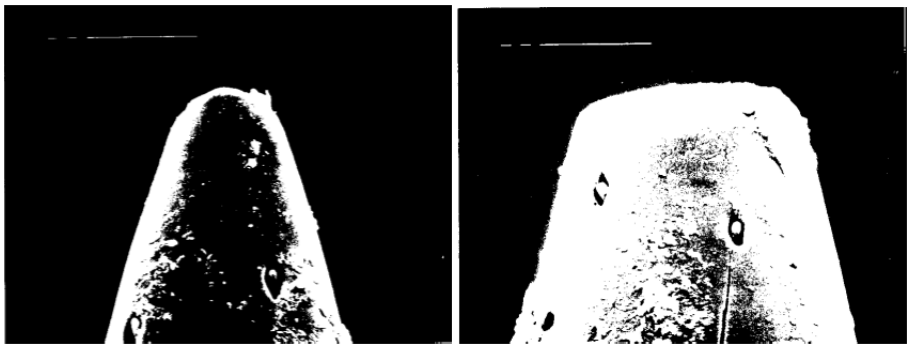


Figure 1.3 Scanning electron micrographs produced by Jones and Kim (1994), depicting (from left to right) the unworn and heavily worn tips of aculei taken from *C. capitata*. Bar, 10 μm.

1.4.3 Physiological and Behavioural Adaptations in Response to Ovipositor Wear

Although many recent studies into insect oviposition have concentrated on physiological factors that influence oviposition choice and behaviour, the importance of an insect's age and physical wear is still regarded as an important issue (Tammaru & Javois, 2005; Schofield *et al*., 2011). Specifically, the strong selective pressures imposed by cuticle wear are believed to have selected for different 'strategies' which either minimize or avoid wear altogether (Lalonde & Mangel, 1994; Schofield *et al*., 2009; 2011). In the following section I concentrate on two such mechanisms; the addition of transition metals and the reuse of conspecific ovipunctures.

1.4.3.1 Increased cuticular hardness through the addition of transition metals

The presence of transition metals in arthropod cuticle, concentrated in mandibles, mouth hooks, claws and ovipositors, has been linked to increased wear resistance (Hillerton & Vincent, 1982; Hillerton *et al*., 1982; Fontaine *et al*., 1991; Quicke *et al*., 1998; Schofield, 2001; Schofield *et al*., 2002, 2003 Vincent & Wegst, 2004; Lichtenegger *et al*., 2008; Morgan *et al*., 2003; Schofield, 2005; Cribb *et al*., 2008a).

When present, such elements are localised in areas where the structure comes into frequent contact with the external environment, such as the cutting edges of mandibles (Hillerton & Vincent, 1982; Morgan *et al*., 2003; Cribb *et al*., 2008b). Zinc-enriched mandibles have been shown to be significantly harder than their unenriched counterparts, with increases in hardness recorded from 20% to more than a two-fold increase, strongly suggesting that zinc-enriched cuticle will experience less wear (Schofield *et al*., 2002; Cribb *et al*., 2008ab; Cribb *et al*., 2010). In contrast, manganese-enriched cuticle does not demonstrate comparable increases in hardness, leading to speculation that it performs different roles (Quicke *et al*., 1998; Morgan *et al*., 2003; Cribb *et al*., 2010).

It has been proposed that the aculei of ovipositing insects may also be enriched with transition metals in order to prevent abrasive wear (Vilhelmsen & Turrisi, 2011). In a study of 57 species of Hymenoptera, Quicke *et al*. (1998) discovered that wasps which used their ovipositors to drill through hard substrates often possessed ovipositors that incorporated the transition metals zinc or manganese, whereas wasps which did not penetrate hard surfaces often contained no traces of these metals. Nevertheless, the performance of unenriched cuticle may be better than previously thought. The unenriched mandibles of the larval jewel beetle, *Pseudotaenia frenchi* (Coleoptera: Buprestidae) displayed a degree of hardness that compared favourably to some stainless steels, and was considerably harder than some adult beetle mandibles enriched with manganese (Cribb *et al*., 2010). Such a result clearly demonstrates that unenriched cuticle is not necessarily softer than enriched cuticle.

1.4.3.2 Damaged host peel and superparasitism

The reuse of ovipunctures, witnessed in some species of tephritid fruit flies, is typically viewed as a subset of insect-host superparasitism. Superparasitism is most studied as a behaviour in which hymenopteran parasitoids deposit their eggs (or a single egg) into hosts that already bear conspecific eggs or larvae (van Alphen & Visser, 1990; Lalonde & Mangel, 1994; Dorn & Beckage, 2007). However, while much of the literature cited in this section relates to parasitoids, the theoretical research can be, and is, applied to repeated use of a single fruit piece by fruit flies.

Two distinct modes of superparasitism have been identified. Self-superparasitism occurs when multiple egg clutches are laid by the same female on an individual host, whereas conspecific superparasitism refers to clutches of eggs laid by different conspecific females (van Alphan & Visser, 1990; Dorn & Beckage, 2007; Gonzalez *et al*., 2007). The traditional view of superparasitism is that it is a purely maladaptive behaviour avoided by ovipositing insects in order to reduce the risk of lethal intraspecific larval competition, which strongly selects for solitary females to lay single-egg clutches and avoid using parasitised hosts (Papaj & Messing, 1996; Dukas *et al*., 2001; Kanno & Harris, 2002; Gonzalez *et al*., 2007). Additional negative effects associated with superparasitism are reduced offspring size and fecundity, due to trade-offs made between the number of offspring reared from the host and the hosts' size (van Alphen & Visser, 1990; Kano & Harris, 2002; Nufio & Papaj, 2004).

Despite the considerable disadvantages associated with superparasitism, there is mounting evidence that superparasitism may confer important fitness advantages upon larvae and adults under appropriate circumstances (van Alphen & Visser, 1990).

Time and egg limitations, coupled with low host and high conspecific female densities, may account for the presence of superparasitism within parasitoid populations (van Alphen & Visser, 1990; Papaj & Messing, 1996). Time-limited wasp parasitoids may superparasitise hosts when host density is relatively low compared to parasitoid density (van Alphen, 1990; Dorn & Beckage, 2007), while an increase in female egg load may also increase the tendency of wasps to superparasitise hosts (Montoya *et al*., 2013). Thus while the offspring from superparasitised hosts typically display signs of reduced fitness, adult fitness may be enhanced through an increase in reproductive opportunity (Lalonde & Mangel, 1994; Nufio & Papaj, 2004).

Adult fruit flies may also gain fitness advantages not simply by superparasitising host fruit, but more specifically by reusing the ovipunctures made by first-comer conspecifics (Nufio & Papaj, 2004). By reusing ovipunctures (or areas of peel that have received damage independent of conspecific oviposition activity) female fruit flies will save time and energy exploiting hosts, and decrease the risks of predation and aculeus damage (Papaj *et al*., 1989b; Lalonde & Mangel, 1994; Papaj & Alonso-Pimentel, 1997; Nufio & Papaj, 2004). These advantages have been suggested as reasons why gregarious walnut husk flies (*Rhagoletis suavis* group) prefers to superparasitise hosts, contradicting the prior belief that female flies will consistently prefer unused hosts when given a choice (Lalonde & Mangel, 1994; Papaj & Messing, 1996; Nufio & Papaj, 2004). The reuse of pre-existing ovipunctures by female fruit flies is thought to represent a trade-off between benefits that enhance adult fitness and the costs associated with increased larval competition (Papaj & Alonso-Pimentel, 1997). Superparasitism may also allow larvae to use unripe fruit as the increased metabolic heat produced by large numbers of larvae will promote bacterial decay,

which in turn will detoxify harmful chemical compounds (Diaz-Fleischer & Aluja, 2003c; Rattanapun *et al*., 2009).

1.5 The Study Organism – *Bactrocera tryoni* (Froggatt)

This project will use the Queensland fruit fly, *Bactrocera tryoni* (Froggatt), as a model system to explore a selection of behaviours to understand the processes, rather than the patterns, of host range use in this fly. *Bactrocera tryoni* is the most economically significant fruit fly pest species in Australia (Fletcher, 1987) and is distributed along the eastern coast of Australia; it has also been recorded as an invasive in New Caledonia, Austral islands, and Easter Island (Drew, 1982). The species is traditionally considered as endemic to tropical and subtropical Australian east coast rainforests, where many of its native hosts are found, but its distribution now encompasses much of Australia's eastern seaboard and inland areas (Drew, 1989; Zamek *et al*., 2012). This range expansion is thought to have been facilitated by increased agricultural production following European settlement (Dominiak & Daniels, 2012).

Bactrocera tryoni was selected as the study organism of this thesis for a number of reasons. The primary characteristic that determined its use was its extremely large host range (Hancock et al. 2000), making it an ideal organism to explore the effects of host peel penetrability upon tephritid oviposition behaviour and host-use. Its wide distribution and abundance also means that it is relatively easy and convenient to obtain specimens from the field. Finally, the fly's status an economically significant pest species has ensured that some aspects of its host range and oviposition behaviour have been previously studied, providing a scientific foundation upon which to base

my work (Pritchard, 1967; 1969; Fitt, 1984; Balagawi, 2005; 2006; Clarke *et al*., 2011; Muthuthantri & Clarke 2012;).

1.6 Thesis Objectives and Rationale

In my literature review, I have focused on host-use by herbivorous insects and some of the different theoretical and mechanistic explainers for host range. I have identified that host choice in tephritids is a complex area, with differences between fundamental and realised host ranges and with dynamic oviposition 'choices' being made by a fly based on its internal physiology, the hosts available and interactions with other conspecifics. Yet despite the economic significance of tephritid fruit flies, their published host lists are still largely simple lists of plant names, devoid of ecological and behavioural context (Balagawi, 2006). There is thus still much that needs to be done to come to grips with tephritid host-use.

Of particular interest to me is the role of host peel and the interaction between aculeus wear and oviposition behaviour. There is a pervasive assumption in the fruit fly literature that increased fly age and cuticular wear of the ovipositor may further influence host preference patterns (Jones & Kim, 1994; Diaz-Fleischer *et al*., 2001; Aluja & Mangan, 2008), and this built upon observations of flies having difficulty in ovipositing into tough-peeled fruit (Messina *et al*., 1991; Balagawi *et al*., 2005; Dhillon *et al*., 2005) and showing behaviours which seem designed to decrease ovipositor wear (Papaj *et al*., 1989; Lalonde & Mangel, 1994; Papaj & Alonso-Pimentel, 1997; Nufio & Papaj, 2004). The problem, however, is that no work has been performed to quantify the effects of aculeus wear upon tephritid oviposition

behaviour, or if such wear does change host range; thus the assumptions that aculeus wear will impact host-use are just that - assumptions.

Given this background, the over-arching hypotheses of this thesis is that host-use by a polyphagous tephritid, *B. tryoni*, will be modified by the physical wear experienced by the ovipositor. More specifically, specific sub-hypotheses are that: (i) wear will occur in the aculei of *B. tryoni* over time and will be proportional to increasing host toughness; (ii) that the host preference patterns and oviposition behaviours of flies with worn aculei will be different to flies with unworn aculei; and (iii) that selection pressures arising from a worn aculeus will result in behavioural and/or physiological mechanisms that mitigate or avoid aculeus wear.

1.6.1 Thesis Structure

The Introduction (Chapter 1) reviews the relevant theoretical and empirical studies related to oviposition behaviour and host range among tephritid fruit flies. I also review the literature pertaining to aculeus wear and its potential to limit realised host range, and the various mechanisms believed to minimise or avoid aculeus wear, and set the outline of the research chapters.

There is general agreement among authors that tephritid aculei undergo progressive wear as a function of fly age and oviposition activity. This has been partially confirmed by Jones & Kim (1994), who noted that the aculei of field caught fruit flies were more worn than laboratory bred flies, however no specific experiments relating increasing ovipositor wear with increasing age have ever been undertaken. In Chapter 2 I test the hypothesis that the aculei of flies exposed to natural hosts of different peel

toughness, or agar fruit mimics of different density, will experience aculeus wear over time, proportional to the type of host to which they were exposed. Over a period of seven weeks I examined the degree of cuticle wear in *B. tryoni* exposed to a range of natural fruit hosts and agar fruit mimics which represented different peel properties (i.e. penetration resistance, elasticity and thickness) or density (i.e. low, medium, or high), respectively. The results confirmed wear occurred, but not in a pattern which might have been predicted.

In Chapters 3 and 4 I explored the issue of host ranking, peel properties and offspring performance. Previous behavioural studies examining the host preference patterns of tephritid fruit flies, including *B. tryoni,* have noted that flies rank host preferentially according to ease of peel penetration (Balagawi *et al*., 2005; Sharma & Amritphale, 2008). For example, Balagawi (2006) noted that *B. tryoni* and *B. cacuminis* (French) exhibited an initial preference for soft-peeled hosts in comparison to hard-peeled hosts across different plant families. However, other studies have shown that fruit flies may select hosts for oviposition which maximise off-spring performance (Greany *et al*., 1985; Messina & Jones, 1990; Diaz-Fleischer & Aluja, 2003b). There is thus conflict in such cases between theoretical predictions made under a preference-performance model of host utilisation, and the physical limitations of oviposition. To test the relative importance of host quality for offspring versus peel penetrability for *B. tryoni* oviposition 'decisions', in this chapter I carried out choice and no-choice oviposition tests using hosts of divergent peel properties and host larval quality.

The potential inability to access hosts brought about by a heavily worn aculeus is thought to be sufficient enough selective pressure to promote the adoption of

mechanisms to minimise or reduce wear. The experiments presented in Chapters 5 and 6 explore two different mechanisms by which this might be done: the physical mechanism of including transition metals in the aculeus cuticle (Chapter 5); and the behavioural mechanism of using of hosts with damaged peel (Chapter 6).

The incorporation of transition metals has been linked to increased cuticle hardness among different orders of insects (Schofield *et al*., 2002; Cribb *et al*., 2008ab; Cribb *et al*., 2010). With collaborators, I conducted an investigation (Chapter 5) to identify the presence of transition metals within the aculei of three species of tephritid fruit flies, and correlated the findings with their reported host ranges.

Much of the results of Chapters 2, 3 and 4 suggested that ovipositor wear and peel toughness is not a serious issue for *B. tryoni* host-use. This is in conflict not only with general expectations of tephritid host-use pattern, but specific observations of *B. tryoni* behaviour in which preferences for existing oviposition holes or other fruit wounds are identified (Prtichard, 1969; Sharma & Amritphale, 2008). Given this conflict between my earlier chapter results and the literature, in Chapter 6 I investigated in detail the behaviour of oviposition with respect to peel integrity (or otherwise) and the presence or absence of conspecific eggs and larvae. The results confirm earlier publications that fruit with damaged peel is preferred over fruit with undamaged peel, but I propose that the evolutionary driver is saved handling time for the female, not a mechanism to avoid ovipositor wear. In the same study, I also identified a novel chemical cue which may be used by the female to avoid fruit holding conspecifics larvae.

Chapter 2: Ovipositor aculeus wear in the fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae): the effect of fruit peel characteristics and fruit density

2.1 Introduction

The Tephritidae (Diptera), or the true fruit flies, are a speciose family of herbivorous insects which include some globally important pests of horticulture, including the Mediterranean fruit fly, *Ceratitis capitata* (Weidemann) and the Oriental fruit fly, *Bactrocera dorsalis* (Hendle) (Cornelius *et al*., 2000; Papachristos & Papadopoulos, 2009). The larvae of frugivorous tephritids feed internally in their host fruit, having emerged from eggs which were placed inside that fruit by the parental female (Bateman, 1972; Clarke *et al*., 2011). The evolution of a specialised ovipositor and oviposition behaviours which allow insertion of eggs inside the host have been regarded as "*key innovations"* in the evolution and diversification of the Tephritidae (Diaz-Fleischer *et al*., 2001).

The tephritid ovipositor consists of abdominal segments seven, eight and nine and is formed by three highly modified components: (i) a tubular or conical oviscape; (ii) an elongate, membranous eversible membrane; and (iii) a needle or blade like aculeus which penetrates the fruit skin (Fletcher, 1987; Diaz-Fleischer *et al*., 2001). The physical size of the tephritid ovipositor (with respect to total body size) varies greatly across the Tephritidae and is thought to be an important evolutionary link with the different larval feeding patterns (e.g. seed feeding versus pulp feeding) seen in different tephritid genera (Aluja & Mangan, 2008). The host range of tephritid fruit flies may be restricted by the ability of the animal to penetrate the peel of the host fruit (Aluja *et al*., 2004; Balagawi *et al*., 2005; Rattanapun *et al*., 2009, 2010). While none of this literature specifically discusses the point, one inference which has been drawn from it is that blunting of the ovipositor during the life of the insect, if it

occurs, may reduce oviposition opportunities and host range (Jones & Kim, 1994; Diaz-Fleischer *et al*., 2001; Aluja & Mangan, 2008).

There has been very little work on ovipositor wear in insects. In a comparative system, the mandibles of herbivorous insects have been shown to undergo abrasive wear in relation to age and substratum hardness, negatively affecting feeding efficiency (Arens, 1990; Wallin, 1988; Kohler *et al*., 2000; Roitberg *et al*., 2005; Vincent, 2009). Quicke *et al*. (1998) have speculated that the presence of transition metals in the ovipositor of parasitic wasps is linked with reducing wear. In tephritid flies, specific behaviours such as superparasitism of host fruit (Papaj & Alonso-Pimentel, 1997) and use of existing fruit wounds (Papaj *et al*., 1989b) have been suggested as mechanisms by which flies minimise ovipositor wear. Diaz-Fleischer *et al*. (2001) commented that little is known about aculeus wear within tephritid fruit flies, and I am aware of only one empirical study which has even recorded wear (Jones & Kim, 1994). In their study, Jones and Kim (1994) found 70% of 71 field-collected fruit flies of four species (*Rhagoletis pomonella* (Walsh), *R. mendax* (Curran), *C. capitata* and *B. oleae* (Gmelin)) had clear signs of ovipositor wear, which manifested itself in the forms of tip blunting and reduced length. In contrast, newly emerged *C. capitata* had much sharper, unworn ovipositors.

As in other tephritid fruit flies, gravid *B. tryoni* females use their ovipositors to penetrate the peel of host fruit to deposit their eggs. The inability of adult females to penetrate fruit peel has been found to explain low frequency host-use of 'cherry' tomatoes, over other tomato varieties, in this fruit fly (Balagawi *et al*., 2005),

demonstrating the importance of understanding oviposition behaviour and ovipositor function for understanding host-use in this species.

In this chapter I specifically wished to test if ovipositor wear occurred over the life of the fly. Additionally, because the polyphagous nature of *B. tryoni* host-use means that the fly is likely to use different host species during its life, I also wished to test if different host types modified the type or rate of wear. These questions were addressed by asking: (i) whether ovipositors of field-collected flies displayed evidence of aculeus wear as compared to newly emerged laboratory flies; and (ii) if the ovipositors of laboratory flies experienced wear when exposed to a variety of host fruit and fruit mimics with variable peel and fruit density characteristics. Three experiments were conducted during this study, all of which were intended to identify signs of aculeus wear in *B. tryoni*. The first experiment compared the aculei of wild flies to that of newly emerged flies, and is essentially identical to the study of Jones and Kim (1994) on the Mediterranean fruit fly. Experiments two and three were laboratory-based studies and followed aculeus wear in cohorts of caged flies following oviposition into fruit types of differing peel types, or following oviposition into agar fruit mimics of differing density.

I made the assumption that the aculei of newly emerged *B. tryoni* females would be unworn based upon the results presented by Jones and Kim (1994). Should aculeus wear occur amongst flies exposed to oviposition substrates, their aculei would be significantly shorter and wider than those taken from newly emerged flies. The apex of the aculeus was regarded as the most appropriate location to identify wear, since it would be exposed to the greatest amount of abrasive wear.

2.2 Methods and Materials

2.2.1 Overview of Experiments

Three experiments were conducted during this study, all of which were intended to identify signs of aculeus wear in *B. tryoni*. The first experiment compared the aculei of wild flies to that of newly emerged flies, and is essentially identical to the study of Jones and Kim (1994) on the Mediterranean fruit fly. Experiments two and three were laboratory-based studies and followed aculeus wear in cohorts of caged flies following oviposition into fruit types of differing peel types, or following oviposition into agar fruit mimics of differing density. The agar fruit mimics were modelled on the technique developed by Diaz-Fleischer and Aluja (2003b), who used such mimics to study oviposition behaviour in the tephritid fruit fly *Anastrepha ludens*. Details of each experiment and common methodologies follow.

2.2.2 Common Methods

Laboratory flies

All flies used in laboratory studies were obtained from cultures maintained by the Queensland Government Department of Agriculture, Fisheries and Forestry (QDAFF), Boggo Road Ecosciences Precinct, Brisbane. The cultures were up to 34 generations old, refreshed every two generations with wild material and reared on carrot-based medium (Christenson *et al*., 1956). For use in experiments, pupae were received from QDAFF and the emergent adults held under ambient conditions at the Queensland University of Technology. Adults held during experimental trials had access to sugar, hydrolysed yeast (MP Biomedicals Australasia Pty. Ltd.) and water *ad libitum*.

Aculeus measurements

Aculeus wear was quantified by using morphometric measurements of the ventro-lateral groove, an easily identifiable and permanent feature of *Bactrocera* aculei (Drew 1989).

Female flies used for aculeus measurements were preserved until dissection in 70% ethanol. For measurement, the ovipositor of each specimen was removed from the abdomen at the seventh abdominal segment. The aculeus was then extracted from the rest of the ovipositor after making a longitudinal incision in the eversible membrane (segment 8) and gently pulling the aculeus out using a pair of fine forceps. Each aculeus was permanently mounted under a coverslip on a microscope slide with the ventral side up.

Aculei were observed using a Leica M125 stereomicroscope, with images captured with a Leica DFC – 90 digital camera. The analysis of digital images was accomplished with the Leica Application Suite (version 3.6.0). The following measurements were taken of the aculeus: 1) the width of the aculeus apex measured 15 μm from the very tip; 2) the distance from the tip to the distal (Lii) end of the ventro-lateral groove and 3) the distance from the apex to the proximal (Li) end of the ventro-lateral groove (Fig. 1). The distance from the apex to the distal end of the ventro-lateral groove was used as a measure of aculeus wear.

Thirty flies were preserved in 70% ethanol one day after emergence, in order to provide baseline measurements of aculei that have not experienced any wear.

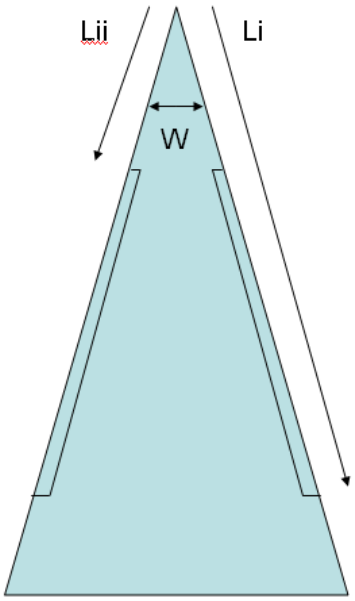


Figure 2.1 Illustrated representation of *Bactrocera tryoni* aculeus apex, displaying the measurements made for the current study. Li = distal end of the ventro-lateral groove; Lii = proximal end of the ventro-lateral groove; W = width measured 15 µm from the tip of the aculeus apex

2.2.3 Methodology of specific experiments

Experiment 1: Comparison of newly emerged and wild

Twenty-nine wild female flies were hand-collected from guava trees in south-east Queensland during December 2010. An additional twenty-one wild female flies were caught using orange-ammonia traps at Mt. Coot-tha during November-December 2012. In Queensland, *B. tryoni* is multivoltine with overlapping generations (Muthuthantri *et al*., 2010) and I presumed the flies were of mixed age. The aculei of

thirty randomly selected wild female flies were compared with those of 30 laboratory flies, placed into alcohol one day after emergence.

Experiment 2: Aculeus wear over time following exposure to different fruit types

Cultured flies were separated into six cages (mesh-sided cages, 33 cm^3), each cage holding a mixed population of 500 female and male flies. From one week after fly emergence, four fruit pieces of the same fruit type were placed into each cage to allow flies to oviposit. The fruit used, apple (*Malus sylvestris* Red delicious), orange (*Citrus cinensis* Navel) and avocado (*Persea americana* Hass) are all regarded as major hosts for *B. tryoni* (Hancock *et al*., 2000) and were purchased as organic fruit from a commercial supplier. Four pieces of the same type of fruit were placed into each cage at random locations. The fruit was kept continually present inside the cages throughout the entire study, with fresh specimens placed inside the cages every 24 hours. Once a week, from Week 1 after emergence through to Week 7 after fly emergence, 10 female flies were removed from the culture and preserved for subsequent aculeus measurement.

Ten fruit pieces for each of the three fruit types were assessed for the peel characteristics elasticity, penetration resistance and thickness. Peel thickness (specifically exocarp thickness) was measured using the Leica equipment on free-hand sectioned, toluene-blue and slide mounted peel cross-sections, with 10 sections cut per fruit piece. Peel toughness was measured using a manual hand-held penetrometer (QA Supplies Model FT 327 Fruit pressure Tester, QA Supplies, Norfolk, USA), which measures the force (N) required to penetrate the fruit using a 2 mm probe in a period of 2 seconds. Three puncture tests per fruit were performed.

Elasticity was measured by calculating the fruit's toughness index (TI) using the average of the three readings for each fruit with the following formula: TI = average force for 2 seconds / (number of peel penetrations out of three attempts + 1). This method was taken from Balagawi *et al*. (2005).

Experiment 3: Aculeus wear over time following exposure to agar fruit mimics of different density

The same experimental protocol was run as for Experiment 2, except real fruit were replaced with agar fruit mimics (sensu Diaz-Fleischer & Aluja, 2003b) of three differing agar densities. The procedure for making the fruit mimics was adapted from Diaz-Fleischer and Aluja and used a mixture of coarse agar powder (10g for soft 'fruit'; 30g medium; and 60g hard), 10g sucrose and 500 mL water, heated to boiling point and poured into hemispherical molds made from bisected table tennis balls where they were allowed to set.

2.2.4 Data Analysis

The means of treatment data from Experiment 1 were compared through one-way analysis of variance (1-way ANOVA) following standard tests for normality (Levene's and Kolmogorov-Smirnov tests) and subsequent Log10 transformation if required. Data sets for Experiments 2 and 3, again following tests for normality and transformation as required, were compared across 'host' and control 'newly emerged flies' treatments (i.e. different fruit or agar densities) using the data collected from the final week of the experiment (days 42-49). The primary logic for this analysis is that I was most interested to test if different oviposition substrates affected wear differentially. My decision for using the data sets from the final week was based on

the expectation that the ovipositor would wear over time. Consequently, ovipositors taken from the end of the study would experience the greatest amount of wear in comparison to ovipositors taken from the beginning of the study. A comparison of newly emerged flies and flies exposed to orange during Week 5 for Experiment 2 was also made. This was done as no observations for the orange treatment past Week 5 could be made because of very heavy losses among the flies within the culture. This result was repeated when the trial was run again with a new culture. Where data could not be normalised, the nonparametric Kruskal-Wallis test was used as an alternative. Tukey post hoc pairwise analysis was performed on data sets conforming to the assumption of a one – way ANOVA, while the Games-Howell post hoc analysis was used as an alternative for data sets that were normally distributed, but did not have equal variance. All tests were conducted with a confidence interval of 95% and results are presented as the mean ± S.E.

2.3 Results

2.3.1 Experiment 1: Comparison of newly emerged & wild flies

There was a significant difference in aculeus length (i.e. Lii length measurement) between laboratory cultured flies and those caught at Cleveland or Mt. Coot-tha (H = 37.416; P = <0.001). The aculei of newly emerged flies were longer (76.970 µm ± 1.284) than flies collected from Cleveland (60.253 µm ±1.836) or Mt. Coot-tha (65.862 µm ± 1.783). A significant difference in aculeus apex width was also detected (H = 16.082; P = <0.001). The aculei of flies collected at Cleveland and Mt. Coot-tha were wider than newly emerged flies (respectively, 17.644 µm ± 0.424, 16.014 µm ± 0.353 and 15.906 µm ± 0.200).

2.3.2 Experiment 2: Aculeus wear following exposure to different fruit types

Exposure to real fruit had a significant impact upon the physical dimensions of *B. tryoni* aculei. Aculeus width changed significantly in response to fruit treatment, whereas aculeus length did not (respectively, $F_{2,25} = 3.736$, $P = 0.038$; $H = 4.603$, $P = 0.100$) (Fig. 2.2ab). The aculei of flies exposed to apple were longer in comparison to flies exposed to orange and avocado (respectively, 71.890 µm ± 1.995, 70.107 µm ± 1.717, 67.634 µm ± 1.920), although these differences were not significant. Post-hoc analysis revealed that the aculeus width of flies exposed to apple was significantly different compared to flies exposed to orange (G/H = 1.531; $P = 0.005$). The aculei of flies exposed to apple were the least wide in comparison to flies exposed to orange and avocado (respectively, 15.361 µm ± 0.230, 16.892 µm ± 0.339, 17.120 µm ± 0.760). No significant linear relationship was found between aculeus width and treatment ($r^2 = 0.192$; $P = 0.20$). No significant linear relationship was found between aculeus length and treatment ($r^2 = 0.091$; $P = 0.119$). It must be noted that although I intended to sample flies of each treatment over a period of seven weeks, it proved impossible to maintain multiple fruit fly colonies continuously exposed to orange for longer than four weeks. Colonies of flies exposed to apple and avocado were maintained for the full seven week period, as did flies which were exposed to agar fruit mimics. I made the decision to compare the shorter lived fly populations exposed to orange alongside flies exposed to apple and avocado in the interest of consistency.

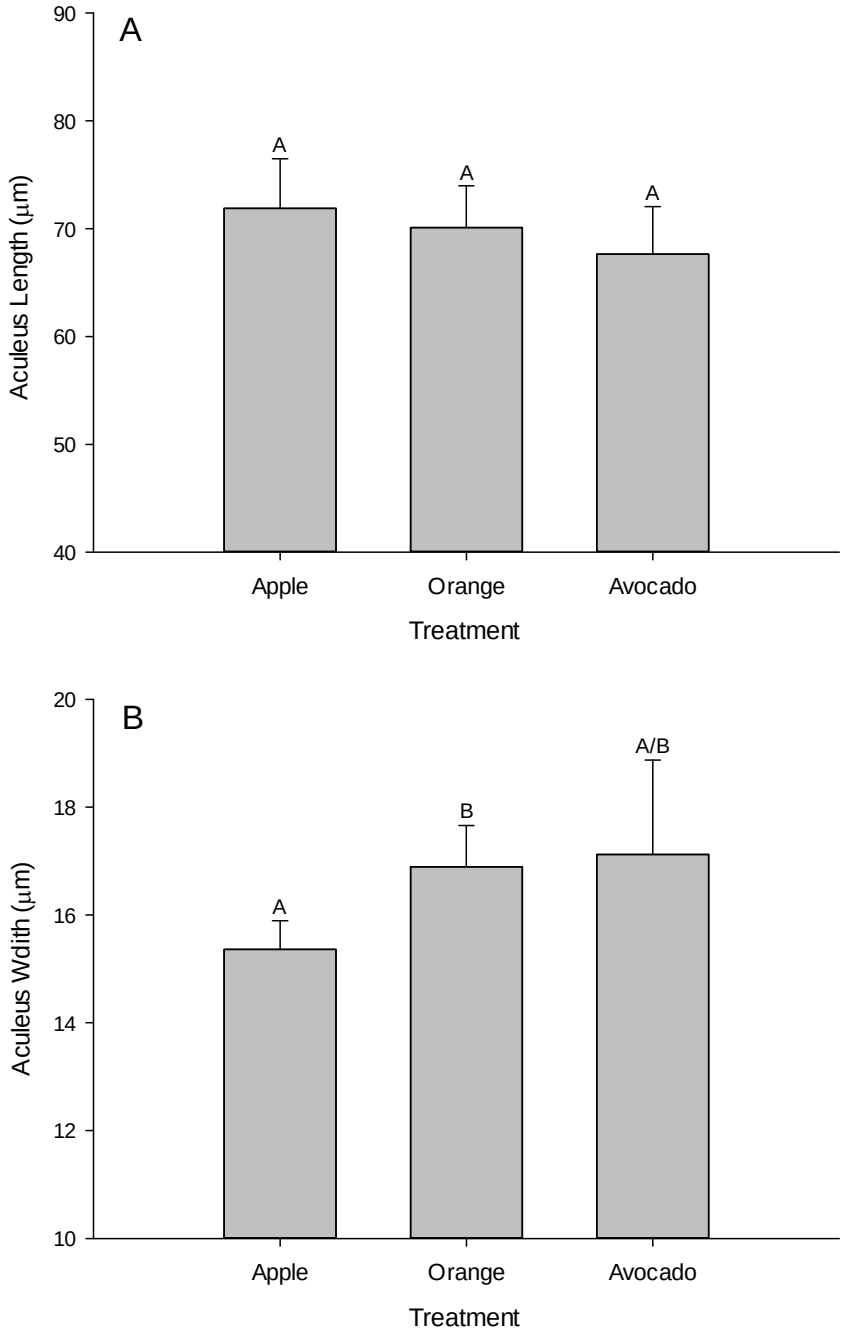


Figure 2.2 Mean (± S.E.) of aculeus apex length (A) and aculeus apex width (B) for female *B. tryoni* continuously exposed to natural host fruit (i.e. apple, orange and avocado) for 49 days. Apple is defined as a soft-peeled host, orange as intermediate and passionfruit as a hard-peeled host. Columns surmounted with the same letter are not significantly different at $\alpha = 0.05$. Aculeus length is determined by measuring the distance from the distal end of the ventro-lateral groove to the apex of the aculeus. The width of the aculeus apex was measured 15 micrometres (µm) from the very tip of the structure.

The length and width of *B. tryoni* aculei did not alter significantly over time (respectively, $F_{7,74} = 1.431$, $P = 0.206$; $F_{7,74} = 0.614$, $P = 0.743$). Linear regression analysis did not find a significant relationship between time and aculeus length or width (respectively, $r^2 = 0.063$, $P = 0.072$; $r^2 = 0.000$, $P = 0.995$).

The length and width of *B. tryoni* aculei exposed to orange altered significantly over time (respectively, $F_{4,61} = 6.032$, $P < 0.001$; $H = 9.496$, $P = 0.050$) (Fig. 2.3ab). Post-hoc analysis found that the aculeus length of newly emerged flies was significantly different compared to flies sampled at Weeks 1, 2, 3 and 4 (respectively $M = 6.826$, $P = 0.028$; $M = 7.878$, $P = 0.008$; $M = 7.148$, $P = 0.028$; $M = 6.862$, $P = 0.019$). Additional post-hoc analysis revealed significant differences in aculeus width between newly emerged flies and flies sampled at Week 4 ($U = 228.000$; $P = 0.014$) and between flies sampled at Week 1 and Week 4 ($U = 82.000$; $P = 0.002$). Strong linear relationships between time and aculeus length and aculeus width (respectively, $r^2 = 0.190$, $F = 14.998$, $P < 0.001$; $r^2 = 0.070$, $F = 4.843$, $P = 0.031$) were detected.

The length of *B. tryoni* aculei exposed to avocado changed significantly over time, whereas no significant changes in aculeus width were observed (respectively, $H = 29.238$, $P < 0.001$; $F_{6,54} = 2.098$, $P = 0.068$) (Fig. 2.3ab). Post-hoc comparisons found that the aculeus length of newly emerged flies was significantly different compared to flies sampled at Weeks 4, 5, 6 and 7 (respectively, $U = 58.000$, $P = 0.004$; $U = 57.000$, $P = 0.004$; $U = 49.000$, $P = 0.004$; $U = 36.000$, $P = 0.001$). Significant differences in aculeus length were also found between Week 1 and Weeks 3 and 7 (respectively, $U = 70.500$, $P = 0.037$; $U = 16.000$, $P = 0.031$), Week 2 and Week 7 ($U = 4.000$; $P = 0.031$) and finally between Week 3 and Weeks 4, 5, 6 and 7

(respectively, $U = 17.500$, $P = 0.014$; $U = 14.000$, $P = 0.006$; $U = 13.000$, $P = 0.009$; $U = 11.000$, $P = 0.005$). Strong linear relationships between time and aculeus length and width were detected (respectively, $r^2 = 0.138$, $P = 0.003$; $r^2 = 0.164$, $P = 0.001$).

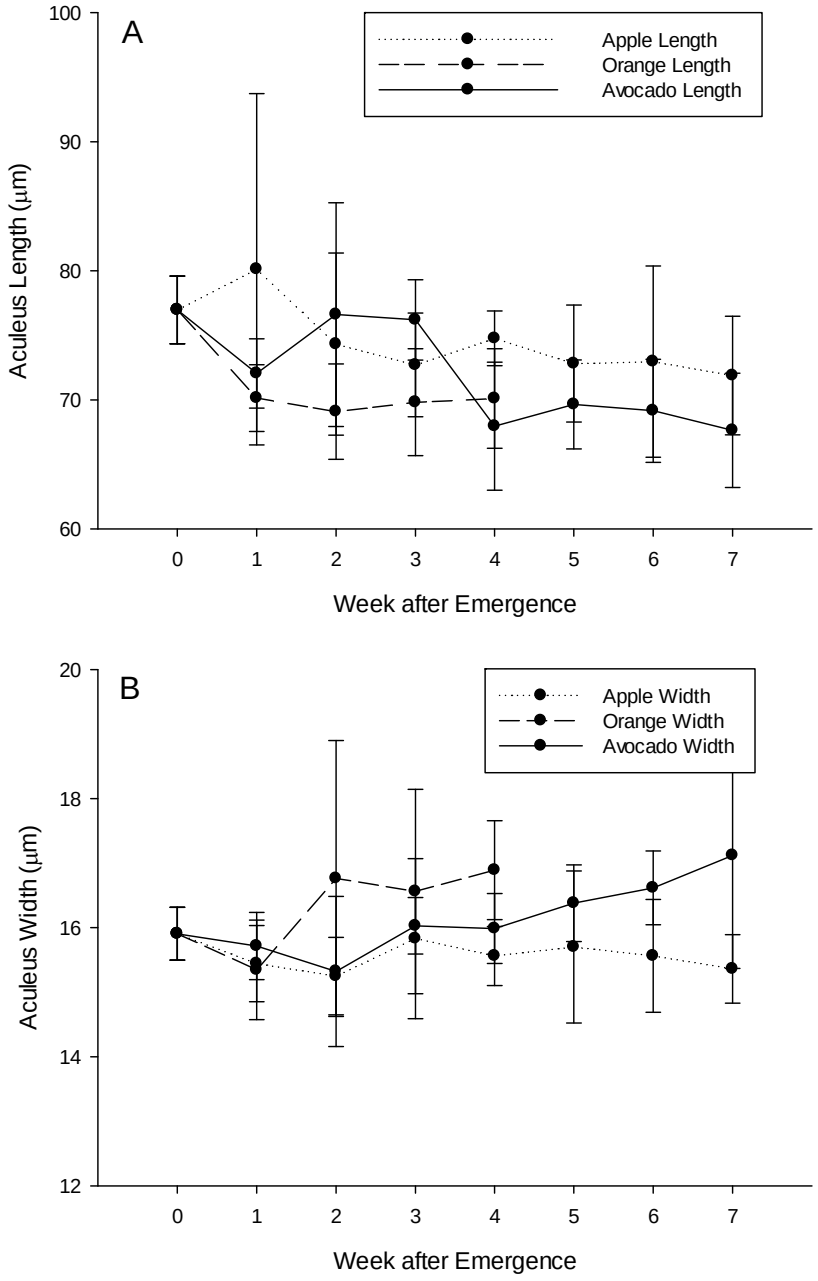


Figure 2.3 Comparisons of aculeus apex length (A) and apex width (B) for newly emerged flies and flies exposed to natural host fruit (i.e. apple, orange and avocado) over weekly intervals (i.e. weeks 1-7). Aculeus length is determined by measuring the distance from the distal end of the ventro-lateral groove to the apex of the aculeus. The width of the aculeus apex was measured 15 micrometres (µm) from the very tip of the structure.

2.3.3 Experiment 3: Aculeus wear following exposure to agar fruit mimics of different densities

I observed significant changes in the length and width of *B. tryoni* aculei exposed to agar fruit mimics of different densities (respectively, $F_{2,27} = 5.242$, $P < 0.012$; $F_{2,27} = 18.555$, $P < 0.001$) (Figure 2.4). Post-hoc analysis revealed that aculeus length for flies exposed to low and high-density fruit mimics was significantly different ($M = 7.755$; $P = 0.009$). The aculei of flies exposed to low-density mimics were the shortest, followed by medium and high-density fruit mimics (respectively, 64.590 µm ± 1.740, 69.228 µm ± 1.897, 72.345 µm ± 1.444). Post-hoc analysis revealed that the aculeus width of flies exposed to low-density fruit mimics was significantly different compared to flies exposed to medium and high-density fruit mimics (respectively, G/H = 2.996, $P = 0.002$; G/H = 3.085, $P = 0.001$). The aculei of flies exposed to low-density mimics were the widest, followed by flies exposed to medium and high-density mimics (respectively, 19.286 µm ± 0.609, 16.290 µm ± 0.326, 16.201 µm ± 0.219). Linear regression analysis found significant relationships between treatment and aculeus length and width (respectively, $r^2 = 0.276$, $P = 0.003$; $r^2 = 0.437$, $P < 0.001$).

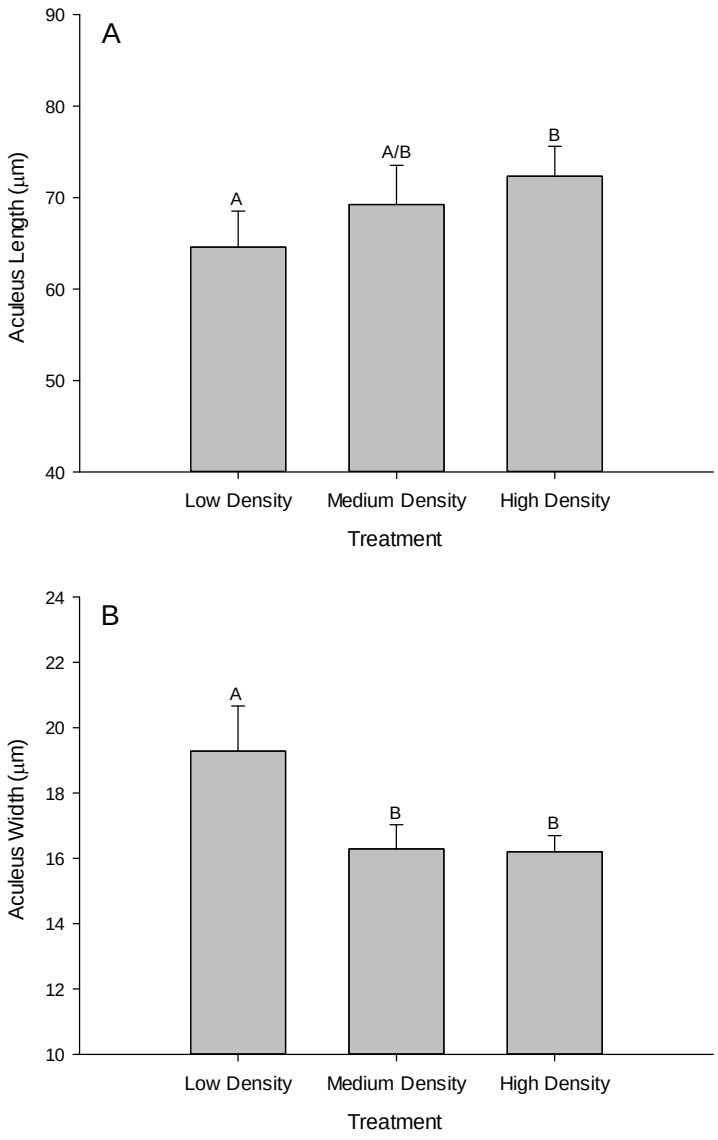


Figure 2.4 Mean (± S.E.) of aculeus apex length (A) and aculeus width (B) for female *B. tryoni* continuously exposed to agar fruit mimics of increasing densities (i.e. low, medium and high) for 49 days. Columns surmounted with the same letter are not significantly different at $\alpha = 0.05$. Aculeus wear is determined by measuring the distance from the distal end of the ventro-lateral groove to the apex of the aculeus. The width of the aculeus apex was measured 15 micrometres (µm) from the very tip of the structure.

The length and width of *B. tryoni* aculei exposed to low-density fruit mimics changed significantly over time (respectively, $F_{7,81} = 7.863$; $P < 0.001$; $F_{7,81} = 6.174$; $P < 0.001$) (Figure 2.5ab). The length of aculei from newly emerged flies was significantly different compared to flies sampled at Weeks 3, 6 and 7 (respectively, G/H = 9.180, $P < 0.001$; G/H = 18.419, $P = 0.005$; G/H = 12.379, $P < 0.001$). The width of aculei from newly emerged flies was significantly different compared to flies sampled at Weeks 6 and 7 (respectively, G/H = 4.766, $P = 0.046$; G/H = 3.380, $P = 0.004$). Strong linear relationships between time and aculeus length and aculeus width (respectively, $r^2 = 0.319$, $P = <0.001$; $r^2 = 0.308$, $P < 0.001$) were detected.

The length of *B. tryoni* aculei exposed to medium-density fruit mimics changed significantly over time, whereas no significant changes in aculeus width were observed (respectively, $H = 20.431$; $P = 0.005$, $H = 12.561$; $P = 0.084$) (Figure 2.5a). The length of aculei from newly emerged flies was significantly different compared to flies sampled at Week 4 ($U = 58.000$; $P = 0.009$). Flies sampled at Week 6 were significantly different compared to newly emerged flies and flies sampled at Weeks 1, 2 and 5 (respectively, $U = 45.000$, $P = 0.001$; $U = 12.000$, $P = 0.025$; $U = 22.000$, $P = 0.034$; $U = 19.000$, $P = 0.019$). Flies sampled at Week 7 were significantly different compared to newly emerged flies and flies sampled at Week 1 (respectively, $U = 59.000$, $P = 0.004$; $U = 13.000$, $P = 0.032$). A strong linear relationship between time and aculeus length was identified, but not between time and aculeus width (respectively, $r^2 = 0.160$; $P = <0.001$, $r^2 = 0.006$; $P = 0.462$).

The width *B. tryoni* aculei exposed to high-density fruit mimics changed over time, whereas no significant changes in aculeus length were observed (respectively, $H =$

18.381; $P = 0.010$, $H = 6.380$; $P = 0.382$) (Figure 2.5ab). The width of aculei from newly emerged flies was significantly different compared to flies sampled at Weeks 2 and 3 (respectively $U = 190.000$, $P = 0.012$; $U = 50.500$, $P = 0.034$). Flies sampled at Week 1 were significantly different to those sampled at Week 2 ($U = 64.000$; $P = 0.007$). Flies sampled at Week 2 were significantly different compared to those sampled at Weeks 3, 4, 5 and 6 (respectively, $U = 1.000$, $P = 0.002$; $U = 13.000$, $P = 0.027$; $U = 10.000$, $P = 0.008$; $U = 16.000$; $P = 0.033$). Flies sampled at Week 3 were significantly different compared to those sampled at Weeks 6 and 7 (respectively, $U = 58.500$, $P = 0.021$; $U = 64.000$, $P = 0.004$). Finally, flies sampled at Week 5 were significantly different compared to those sampled at Week 7 ($U = 78.500$; $P = 0.030$). Time had a significant linear relationship with aculeus length, although not with aculeus width (respectively, $r^2 = 0.060$, $P = 0.018$; $r^2 = 0.004$, $P = 0.542$).

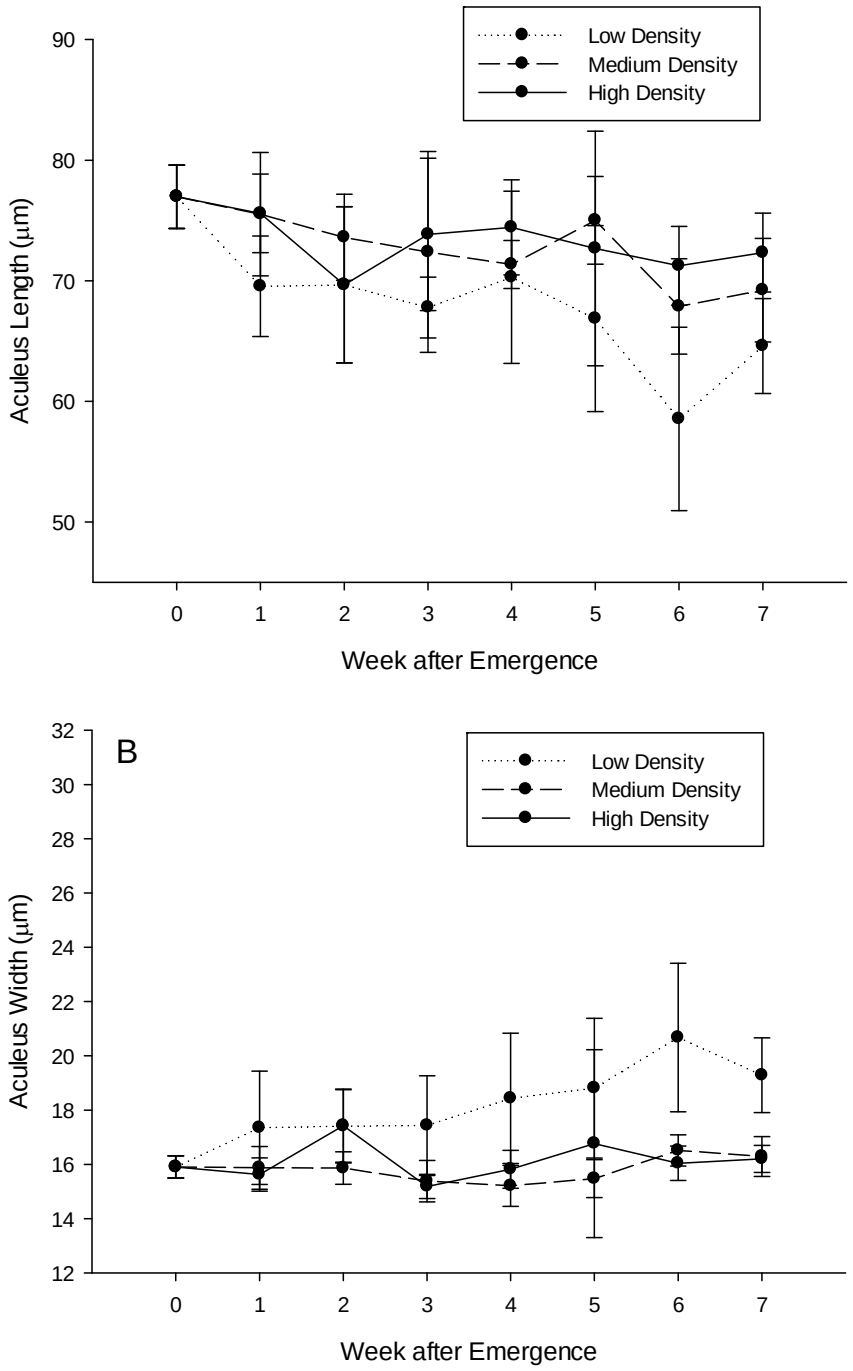


Figure 2.5 Comparison of aculeus apex length (A) and apex width (B) for newly emerged flies and flies exposed to artificial fruit mimics (i.e. low, medium and high densities) over weekly intervals (i.e. weeks 1-7). Aculeus length is determined by measuring the distance from the distal end of the ventro-lateral groove to the apex of the aculeus. The width of the aculeus apex was measured 15 micrometres (µm) from the very tip of the structure.

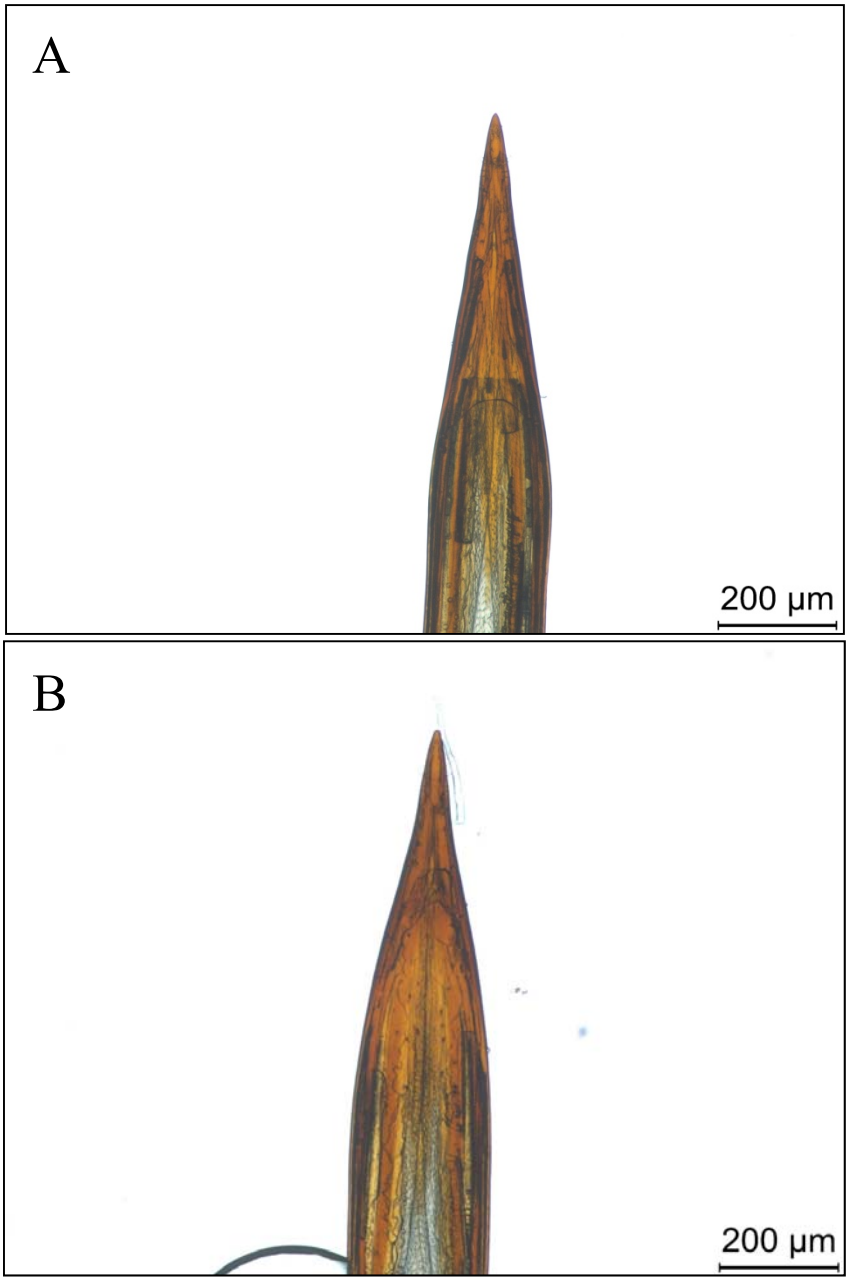


Figure 2.6 Light microscope images of *B. tryoni* aculei tips exposed to avocado at Week 1 (A) and Week 7 (B).

2.4 Discussion

The aculei of flies exposed to agar fruit mimics exhibited clear and consistent patterns of wear, although not in the manner I expected. Aculeus wear is defined as a decrease in apex length, and a concomitant increase in width as the structure becomes shorter through abrasion. I hypothesised that if aculeus wear did occur, it would be most prevalent in the aculei of flies exposed to orange and avocado (respectively, intermediate and hard-peeled hosts) and high-density agar mimics as opposed to flies exposed to soft-peeled apple and low-density mimics, or flies which had been preserved one week after emergence. I further hypothesised that *B. tryoni* aculei would become more worn as time progressed. The first experiment compared the aculei of newly emerged laboratory flies, which were not given the opportunity to oviposit, with field-collected flies for signs of aculeus wear. In experiments two and three, flies were exposed to a variety of host fruit and agar mimics with variable peel and density characteristics over a period of seven weeks. In conducting the three experiments I could determine the effects of different oviposition substrates and time upon aculeus wear.

The aculei of flies exposed to low-density mimics were considerably shorter than those exposed to medium and high-density mimics. Furthermore, the aculei of flies exposed to low-density mimics were considerably wider than those exposed to medium and high-density mimics. Again, these results did not conform to my expectations. The pattern of wear displayed by flies exposed to natural host fruit bore a greater resemblance to the patterns described by a range of different researchers (Wallin, 1998; Arens, 1990; Dockter, 1993; Kholer *et al*., 2000). Although the aculei

of flies exposed to apple were slightly longer in comparison to those exposed to orange and avocado, the lack of meaningful differences makes it difficult to speculate how the three relevant fruit peel properties contribute towards aculeus wear. In regards to aculeus width, the aculei of flies exposed to apple were the narrowest, and the aculei of flies exposed to orange and avocado becoming progressively wider.

B. tryoni aculei show signs of wear over time. The clearest pattern was found among flies exposed to orange, in which newly emerged flies were significantly more worn and narrower compared to flies sampled in Week 4. The same pattern was also found for flies exposed to low-density fruit mimics, in which the aculei of newly emerged flies were less worn and narrower than flies from Weeks 6 and 7. This is the first time that aculeus wear in fruit flies has been tracked over time, and these results lend weight to the belief that the aculei of female tephritids become increasingly worn over their lifetime (Okolle & Ntonifor, 2005; Kim & Jones, 1994).

Although the aculei of flies exposed to orange and low and high-density fruit mimics became significantly wider over time, the heavily blunted aculei observed in *C. capitata* (Jones & Kim, 1994) was not duplicated in our results. A pointed aculeus apex was maintained in all of the specimens I observed. The clear difference in wear was unexpected due to the similarities in aculeus morphology between *C. capitata* and *B. tryoni* (White, 2001) and the assumed importance of morphology differentiating monophagous and polyphagous species (Jones *et al*., 1993; Balagawi *et al*., 2005; Sayar *et al*., 2009). Recent investigations into species of *Bactrocera* and *Ceratitis* have revealed noticeable differences in ovipositor musculature which the authors speculate could be related to their ability to effectively use host fruit

(Ovtshinnikova, 2012). Consequently, there may well be other important differences in aculeus structure between *B. tryoni* and *C. capitata* that are responsible for the different patterns of wear.

My first explanation for this discrepancy between the observed and anticipated pattern of wear is that the heavily worn specimen of *C. capitata* presented by Jones & Kim (1994) represents an extremely old individual. Adult tephritids with wide host ranges may be relatively long-lived (Brevault, *et al*., 2008), although longevity is also influenced by a range of abiotic and biotic factors (Fanson *et al*., 2009; Mohd Noor *et al*., 2011). Observations by other researchers have seen fruit flies live for more than 100 days, although such examples only occurred in studies where specimens were maintained under optimal laboratory conditions (Krainacker *et al*., 1987; Meksonngsee *et al*., 1988; Dhillon *et al*., 2005; Yokoyama, 2012). Furthermore, cuticular wear was apparent after 21 days in both the meadow grasshopper *Chorthippus parallelus* (Zett.) and the true bug *Dicyphus hersperus* (Köhler *et al*., 2000; Roitberg *et al*., 2005). If pronounced abrasion was apparent within a similar time period between representatives of two separate arthropod lineages, I consider it unlikely that a heavily abraded aculei apex would not be observed after 49 days of exposure to fruit mimics.

My favoured explanation for this result is an abrasion-resistant apex, possibly due to the incorporations of transition metals including zinc (Zn) and manganese (Mn). The less resistant sides of the aculeus wear away, while the reinforced tip remains intact. Similar examples of self-sharpening cuticular structures have been found in prior studies (Hillerton, 1980; Hillerton *et al*., 1982; Bernays *et al*., 1991; Dunlop & Fratzl,

2010). The aculeus of *C. capitata* may lack such a reinforced tip when compared to *B. tryoni*, leaving it more vulnerable to wear. The incorporation of transition metals within cuticle has been found in the contact regions of cuticular 'tools' including mandibles, mouthhooks, leg claws and ovipositors among numerous species of insects (Hillerton & Vincent, 1982; Hillerton *et al*., 1982; Quicke *et al*., 1998; Schofield *et al*., 2002; Morgan *et al*., 2003; Cribb *et al*., 2010). The presence of these metals has been linked to increased cuticular hardness (Hillerton & Vincent, 1982; Fontaine *et al*., 1991; Quick *et al*., 1998; Schofield *et al*., 2003; Vincent & Wegst, 2004; Broomell *et al*., 2008). However cuticular hardness is not solely dependent upon the presence of transition elements (Cribb *et al*., 2010). Tanning and the presence of water also affect the mechanical properties of cuticle (Cribb *et al*., 2010; Klocke & Schmitz, 2011).

The greater amount of aculeus wear displayed by flies exposed to low-density fruit mimics at first appears counter-intuitive, but for this there is a likely behavioural explanation. Female tephritids generally display a preference for soft fruit over hard fruit (Messina & Jones, 1990; Balagawi *et al*., 2005). It has also been demonstrated that tephritids will lay fewer, but larger clutches of eggs into harder fruit; and more frequent but smaller clutches of eggs into soft fruit (Diaz-Fleischer & Aluja, 2003b; Birke *et al*., 2006; Rattanapun *et al*., 2009). Thus while I did not quantify the number of ovipositions made by flies into the different fruit mimics, I strongly suspect the greater aculeus wear witnessed in flies exposed to soft fruit mimics is probably due to a greater number of oviposition events into these fruit.

Although I observed clear differences in aculeus wear and width between flies exposed to agar mimics of different densities, I found no significant difference between experimental treatments in regards to aculeus wear for flies exposed to natural host fruit. I speculate that the absence of a clear pattern is due to the reuse of ovipositor holes made within the host fruit, a behaviour documented in *B. tryoni* (Bateman, 1972; Diehl & Prokopy, 1986; Messina & Jones, 1990; Nguyen *et al*., 2007), coupled with the high number of flies confined in a limited volume of space using a small number of resources. Alternatively, the differences between the natural host fruit and the agar fruit mimics may be responsible. The density of agar mimics is consistent throughout, as is the force needed to insert the entire aculeus within the mimic unlike natural fruit, whose outer peel is the greatest barrier to penetration (Pritchard, 1969; Messina & Jones, 1990; Jones & Kim, 1994).

The potential impact of aculeus wear upon oviposition behaviour and host range in polyphagous tephritids has been commented upon in the past, but has not been tested with a species in which aculeus wear has been identified. The experiments detailed in Chapters 3 and 4 describe my attempts to identify patterns of oviposition behaviour and host preference in *B. tryoni* exposed to fruit hosts of different penetrability.

Chapter 3: Variation in Host Range in Queensland Fruit Fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) based on Different Fruit Peel Characteristics

3.1 Introduction

Fruit flies (Diptera: Tephritidae) are globally important agricultural pests, with adult females laying their eggs into suitable host fruit and the resultant larvae feeding on the flesh of the hosts (Davies *et al*., 1999; Clarke *et al*., 2005; Clarke *et al*., 2011). A number of tephritid species, such as the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and the Queensland fruit fly, *Bactrocera tryoni* (Froggatt), are highly polyphagous with larval host ranges including species from more than 20 plant families (Hancock *et al*., 2000). The wide host range of polyphagous fruit flies is typically attributed to a complex mix of fly (intrinsic) and host fruit (extrinsic) related factors (Aluja & Mangan, 2008). For example, the ability to locate hosts using signals or cues common to all host plants is considered one intrinsic trait important to polyphagous herbivores, while the production of a common volatile compound may be an extrinsic host associated attribute linking the fruits used by polyphagous insects (Menken, 1996; Rajapakse *et al*., 2006). Once an appropriate host fruit has been selected and the female has alighted upon it, host suitability and oviposition site selection are assessed using sensory receptors located on antennae, mouthparts, tarsi and the ovipositor (Fletcher & Prokopy, 1991; Navrozidis & Tzanakis, 2005; Sharma & Amritphale, 2008). There is increasing evidence that the host range of polyphagous tephritids is partly determined by the interaction between fruit peel properties and the ability of the fly to successfully penetrate through the peel (Diaz-Fleischer & Aluja, 2003b; Rouquette & Davis, 2003; Aluja *et al*., 2004; Balagawi *et al*., 2005; Rattanapun *et al*., 2009; 2010).

Tephritids often have difficulty when attempting to penetrate the skin of host fruit for oviposition (Pritchard, 1969; Messina & Jones, 1990; Jones & Kim, 1994), and there

is strong evidence that tephritids prefer hosts more easily penetrated (Messina *et al*., 1991; Balagawi *et al*., 2005; Rattanapun *et al*., 2009). The ability of fruit peel to resist oviposition by fruit flies has been credited to a number of physical properties including peel thickness and toughness, which may vary across different species of host fruit or within the same species at different levels of ripeness (Pritchard, 1969; Messina *et al*., 1991; Aluja & Mangan, 2008; Papachristos & Papadopoulos, 2009). Examples of fruit peel inhibiting oviposition include the western cherry fruit fly (*Rhagoletis indifferens* Curran), which successfully infested host fruit (tart cherry *Prunus cerasus*) when the mean penetration resistance of the fruit peel declined (Messina *et al*., 1991). Balagawi *et al*. (2005) attributed peel toughness in the tomato (*Lycopersicon lycopersicum*) cultivar Cherry for its low rate of infestation, as did Dhillon *et al*. (2005) for the melon fruit fly (*Bactrocera cucurbitae* Coquillett).

Although peel attributes such as penetration resistance and thickness are often associated with overall 'toughnes', additional peel attributes including peel surface texture and epicuticle wax have also been shown to influence oviposition behaviour (Thorsteinson, 1960; Greany *et al*., 1985; Birke *et al*., 2006; Diaz-Fleischer *et al*., 2001; Sharma & Amritphale, 2008). Fruit surfaces that are exceptionally smooth or oily may deter oviposition (Pritchard, 1969; Balagawi *et al*., 2005; Dhillon *et al*., 2005). The epicuticular wax layer often found on the surfaces of host fruit may inhibit or assist oviposition, depending upon the species of fruit fly (Eigenbrode & Espelie, 1995; Eigenbrode, 2004).

Although peel toughness is seen as a limiting factor for tephritd oviposition behaviour (Seo *et al*., 1982; Neuenschwander *et al*., 1985; Messina & Jones, 1990; Messina *et*

al., 1991; Balagawi *et al*., 2005; Rattanapun *et al*., 2009), there is also evidence that suggests that increased peel penetration resistance and peel thickness are not qualities automatically correlated with a reduction of oviposition (Papachristos & Papadopoulos, 2009). Tephritids may circumvent fruit peel in a number of ways, which can be broadly categorised as morphological or behavioural. Specialised ovipositor morphology is a trait which is considered especially important in helping understand tephritid host range (Diaz-Fleischer *et al*., 2001; White, 2001; Rouqette & Davis, 2003; Sayar *et al*., 2009), with the development of a specialised ovipositor considered a key evolutionary development for the family (Diaz-Fleischer *et al*., 2001). Specialised ovipositor traits believed to contribute to increased host range in tephritids include: increased ovipositor sharpness; number of apical teeth; increased ovipositor hardness; and fusion of tergal rods (Rouqette & Davis, 2003). Some studies have shown significant consistency in ovipositor morphology among functional groups of fruit flies, for example a simple pointed, needle like aculeus was the only ovipositor shape identified among polyphagous pest species in the tribe Dacini, although this was also the most common (but not only) shape within this tephritid clade (White, 2001).

Tephritids also use behaviour to overcome host-use restrictions imposed by fruit peel. To get through peel, fruit flies may use oviposition wounds made by conspecific flies, or may select areas of the peel where mechanical or disease damage has occurred (Bateman, 1972; Diehl & Prokopy, 1986; Papaj *et al*., 1989b; Messina & Jones, 1990; Nguyen *et al*., 2007). It has also been noted that some fruit flies preferentially oviposit at the top of fruit pieces, which may be a mechanical or evolved behavioural response

to oviposit where the peel is softest in gradually ripening (= climacteric) fruit (Rattanapun *et al*., 2010).

While there is substantial evidence that highlights the importance of fruit peel characteristics in host-use by tephritids and allows strong inferences to be made, no study that I am aware of has been explicitly designed to test the presumed link between host fruit penetrability and host range in a polyphagous fruit fly. Most previous studies exploring mechanisms of host-use in polyphagous tephritids have focused on links between adult preference and juvenile performance (e.g. Fitt, 1986b; Rattanapun *et al*., 2010), despite such linkages often being weak for these insects (Diaz-Fleischer *et al*., 2001). In contrast, I specifically wished to test if the realised host range of a polyphagous tephritid is different from its potential host range (sensu Fitt, 1986b) because of mechanical restrictions imposed by the potential host fruit. The highly polyphagous dacine tephritid *B. tryoni* remained the model organism of this study, a fly for which host range is likely restricted by adult rather than larval attributes (Fitt, 1986b), and for which host peel has been previously implicated in influencing oviposition (Pritchard, 1969; Bateman, 1972; Balagawi *et al*., 2005). I used for my trials known host fruit of *B. tryoni* with very different peel characteristics (e.g. penetration resistance; elasticity; thickness), and also agar fruit mimics with different surface characteristics (surface texture; wax layer).

3.2 Materials and Methods

3.2.1 Overview of Experiments

Two laboratory-based experiments were conducted during this study, designed to identify preferences towards oviposition hosts with different peel or surface characteristics. The first experiment was a no-choice study, where small groups of gravid female *B. tryoni* were exposed to different natural host fruits and agar fruit mimics with different peel and surface characteristics. The second experiment examined the same host preferences, but this time in a choice environment. The fruit used (apple, orange, avocado, passionfruit) were chosen based on differences in the peel properties penetrability, elasticity and thickness and are all recorded hosts of *B. tryoni* (Hancock *et al*., 2000); in the field the fly is a commercial pest of these fruits (Swaine *et al*., 1985). Agar fruit mimics, modelled on the technique developed by Diaz-Fleischer (2003b), were used to assess the impact of the surface textural qualities roughness and slipperiness. The purpose of the experiments was to see if fruit properties limited the realised host range of *B. tryoni* (no-choice tests), or modified host usage (choice tests). Details of each experiment and common methodologies follow.

3.2.2 Common Methods

Laboratory flies

All flies used in laboratory studies were obtained from cultures maintained by the Queensland Government Department of Agriculture, Fisheries and Forestry (QDAFF), Boggo Road Ecosciences Precinct, Brisbane. The cultures were up to 34 generations old, refreshed every two generations with wild material and reared on carrot-based medium (Christenson *et al*., 1956). For use in experiments, pupae were received from QDAFF and the emergent adults held under ambient conditions at the Queensland University of Technology. Adults held during experimental trials had *ad*

libitum access to sugar, hydrolysed yeast (MP Biomedicals Australasia Pty. Ltd.) and water.

Oviposition Substrates

Agar fruit mimics were made after adapting the procedure of Diaz-Fleischer (2003b) in order to alter mimic surface characteristics. A mixture of coarse agar powder (10g), sucrose (10g) and water (500 mL) was heated to boiling point and poured into hemispherical molds. Surface smoothness was altered by adding 6 g of vermiculite to the heated agar mixture, prior to pouring it within the moulds. The moulds themselves were also altered, with smooth-surfaced mimics produced in bisected tennis balls, and rough-surfaced mimics produced in dimpled plastic golf balls. In order to approximate the epicuticular wax layer often present on the surfaces of fruit, a small amount of Carnauba Xtra apple wax (Colin Campbell (Chemicals) Pty. Ltd.) was applied to the surface of fruit mimics with a soft-bristled paint brush. Four types of agar fruit mimic were used in Experiments 1 and 2: (i) smooth and unwaxed; (ii) rough and unwaxed; (iii) smooth and waxed; and (iv) rough and waxed.

Four types of host fruit, each from different genera, were used in Experiment 1. The fruit used, apple (*Malus sylvestris* Red delicious), orange (*Citrus cinensis* Navel), avocado (*Persea americana* Hass) and passionfruit (*Passiflora edulis* Panama red) are all regarded as hosts or major host for *B. tryoni* (Hancock *et al*., 2000) and were purchased as organic from a commercial supplier. Fruit whose peel displayed obvious signs of damage were rejected.

Ten randomly chosen ripe pieces for each fruit type were assessed for peel penetration resistance, elasticity and thickness, with the results used to interpret patterns of host-

use. Penetration resistance measurements were made using a penetrometer (QA Supplies Model FT 327 Fruit Pressure Tester, QA Supplies, Norfolk, USA), which measured the force (N) required to penetrate a fruit using a 1 mm diameter probe in a period of two seconds. Three puncture tests per fruit were performed. Elasticity was measured through calculation of the fruit's toughness index (TI) using the average of the three readings for each fruit with the formula: TI = average force for 2 seconds / (number of peel penetrations out of three attempts + 1). Peel thickness (specifically exocarp thickness) was measured using free-hand sectioned, toluene-blue and slide mounted peel cross-sections, with 10 section cut per fruit piece. Peel thickness measurements were carried out using a Leica M125 stereomicroscope with images captured with a Leica DFC-90 digital camera. Image analysis was accomplished with the Leica Application Suite (version 3.6.0).

Behavioural observations

Behavioural studies were performed in three, 2 hour observation blocks between 0800 and 1400 hr. All observations were conducted outside under natural temperature and lighting conditions between August and September 2011. Four cages were used in each observation block. Observations commenced with the introduction of three gravid, ovipositionally-naive female flies into a cage (clear Perspex sided cages, 40x40x40 cm) containing host fruit or agar fruit mimics.

Host fruit preference was measured by the number of fly visitations to the fruit, and oviposition behaviour was classified as the number of oviposition events, the proportion of oviposition attempts that were successful and number of times that previously made ovipunctures were reused. A visitation was defined as an individual

fly maintaining contact with the surface of the fruit for 5 seconds or more. An oviposition attempt was defined as the fly arching its abdomen approximately 60 - 70º with the fruit surface at the point of penetration and attempting to push its ovipositor through the peel. A successful oviposition event was defined as when the ovipositor had clearly penetrated the skin of the fruit and is brought to an angle of 90º with the fruit surface (Pritchard, 1969). At the conclusion of each block of observations both flies and host fruit were replaced with fresh specimens. If no activity was witnessed inside a cage after 20 minutes both the flies and fruit were replaced with fresh specimens.

For no-choice trials (Experiment 1), 12 replications were made of 2 hr observation periods for three flies exposed to a single fruit piece or agar fruit mimic. For choice trials (Experiment 2) the same replication was used, except all four fruit, or all four agar fruit mimic types, were offered simultaneously. Host fruit were placed in the cages at random positions in order to avoid positional effects. For all trials involving fruit, the stem attachment point of the natural host fruit (peduncle) was covered by a piece of adhesive paper. This was done in order to prevent flies from exploiting a vulnerable point of the fruit. I observed such behaviour during preliminary experiments when flies were exposed to passionfruit.

3.2.3 Data Analysis

The means of treatment data from Experiment 1 and Experiment 2 were compared across experimental treatments through one-way analysis of variance (1-way ANOVA) following standard tests for normality (Levene's & Kolmogorov-Smirnov tests) and subsequent Log10 transformations if required. Where data could not be normalised, the nonparametric Kruskal-Wallis test was used as an alternative. Tukey

post-hoc pairwise analysis was performed on data sets conforming to the assumption of a one-way ANOVA, while the Game-Howell post-hoc analysis was used as an alternative for data sets that were normally distributed, but did not have equal variance. All tests were conducted with a confidence interval of 95% and results are presented as the mean ± S.E.

3.3 Results

3.3.1 Peel Properties

I found a significant difference in peel penetration resistance (N) between the four fruit types ($F_{3,116} = 10.797$, $P < 0.001$) (Table 3.1). Apple displayed the greatest resistance to force, followed by avocado, passionfruit and orange. The penetration resistance of apple was significantly greater than passionfruit and orange, but not avocado. Penetration resistance between orange and avocado was also significantly different. Similarly, I detected a significant difference in the elasticity of the peels of fruit used in this study ($F_{3,36} = 66.052$; $P < 0.001$) (Table 3.1). The elasticity of passionfruit peel was significantly greater than apple, orange and avocado, but the latter three fruit were not different from each other. Finally, a highly significant difference in fruit peel thickness was detected ($H = 111.472$; $P < 0.001$), with all fruits differing from each other. Orange had the thicknest peel, followed by passionfruit, avocado and apple (Table 3.1).

Table 3.1 Mean (± S.E.) peel characteristics of the four types of fruit which are known hosts of *Bactrocera tryoni*. Values in the same row followed by the same letter are not significantly different at $\alpha = 0.05$. Fruit peel resistance to penetration was measured by recording the force (N) required to penetrate the fruit using a 2 mm probe in a period of 2 seconds.

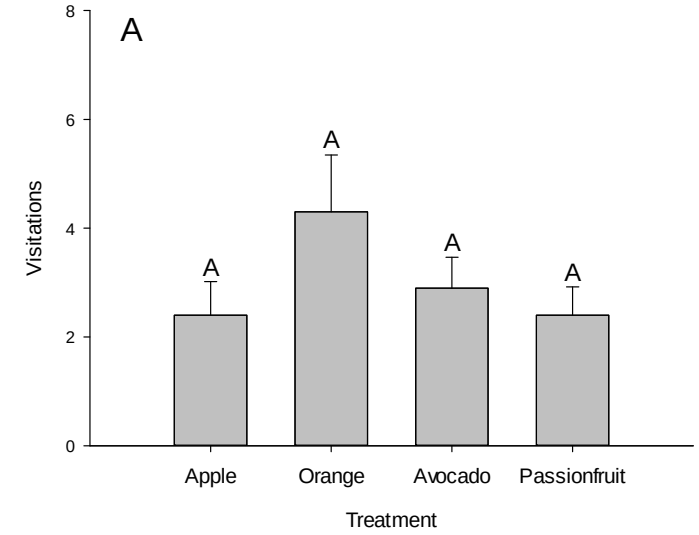
	Apple	Orange	Avocado	Passionfruit
Resistance	1.62 ± 0.06a	1.18 ± 0.05c	1.52 ± 0.05ab	1.39 ± 0.06bc
Elasticity	0.404 ± 0.018a	0.406 ± 0.058a	0.449 ± 0.039a	1.390 ± 0.096b
Thickness	112.75 ± 3.60a	3827.47 ± 71.74d	1035.22 ± 46.85b	2231.22 ± 79.18c

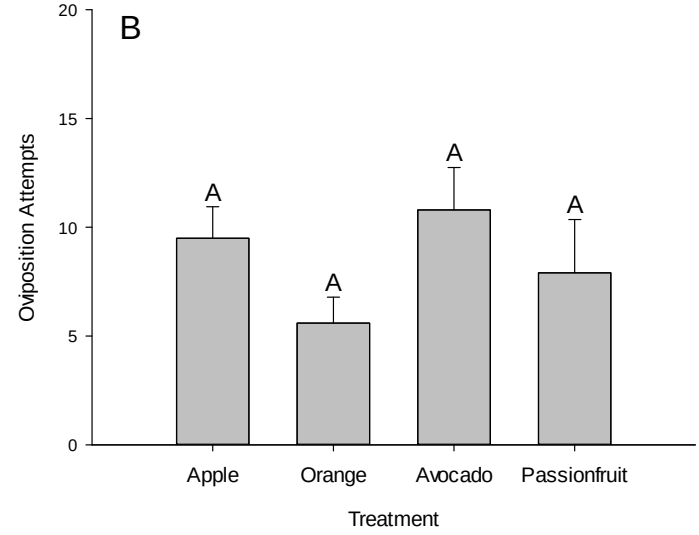
3.3.2 Experiment 1: Host preference and use in no-choice scenarios

Analysis found that the type of oviposition substrate had no impact on the number of visitations when flies were exposed to either natural or agar fruit mimic hosts (respectively, $F_{3,36} = 1.563$, $P = 0.215$; $H = 1.959$, $P = 0.581$) (Fig. 3.1a & 3.2a). This result was repeated for the number of attempted oviposition events (respectively, $H = 5.020$, $P = 0.170$; $H = 1.727$, $P = 0.631$) (Fig. 3.1b & 3.2b). Across all trials, the mean number of visitations to a fruit piece was 2.538 S.D. = 1.948 and to an agar mimic 5.491 S.D. = 4.961, while the mean number of oviposition attempts into fruit and agar mimics was 5.875 S.D. = 5.849 and 1.966 S.D. = 2.731, respectively.

Oviposition substrate had a significant impact upon the number of successful oviposition events when flies were exposed to natural and agar fruit mimic hosts in no choice scenarios (Fig. 3.1c & 3.2c). Most notably, no oviposition into passionfruit was ever observed. When a one-way ANOVA was carried out to compare the remaining fruit (passionfruit was excluded from this analysis), differences were still found ($F_{2,24} = 4.783$; $P = 0.018$), with post-hoc analysis detecting significant differences between orange and avocado, with apple intermediate between the two (Fig. 3.1c). For the agar fruit mimics, there was again a significant difference in successful oviposition between treatments ($H = 8.282$; $P = 0.041$). The smooth waxed and unwaxed mimics had similar rates of successful oviposition (at ~50-55%), and

both were significantly higher than the rough, unwaxed mimic. The rough, waxed mimic was intermediate between these three (Fig. 3.2c). I witnessed three examples of ovipuncture wounds being reused in the rough (waxed) treatment, and a single instance of ovipuncture reuse in the rough (unwaxed) and smooth (unwaxed) treatments.


A
Visitations
8
6
4
2
0
A
A
A
A
Apple
Orange
Avocado
Passionfruit
Treatment


B
Oviposition Attempts
20
15
10
5
0
A
A
A
A
Apple
Orange
Avocado
Passionfruit
Treatment

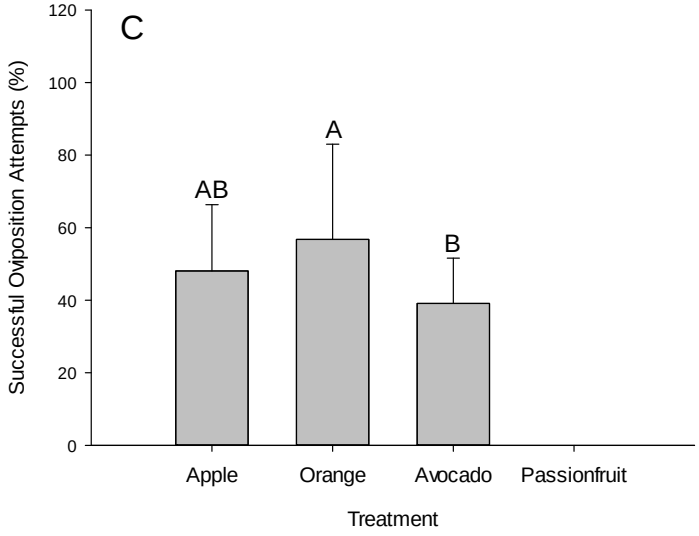
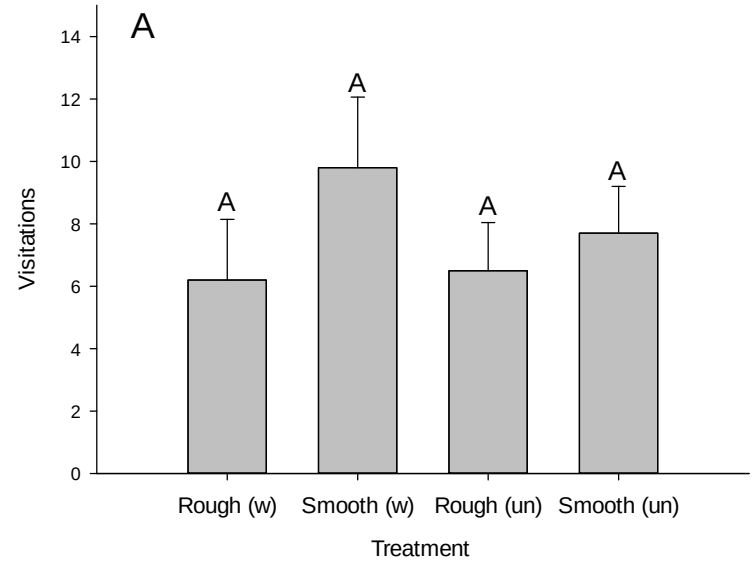
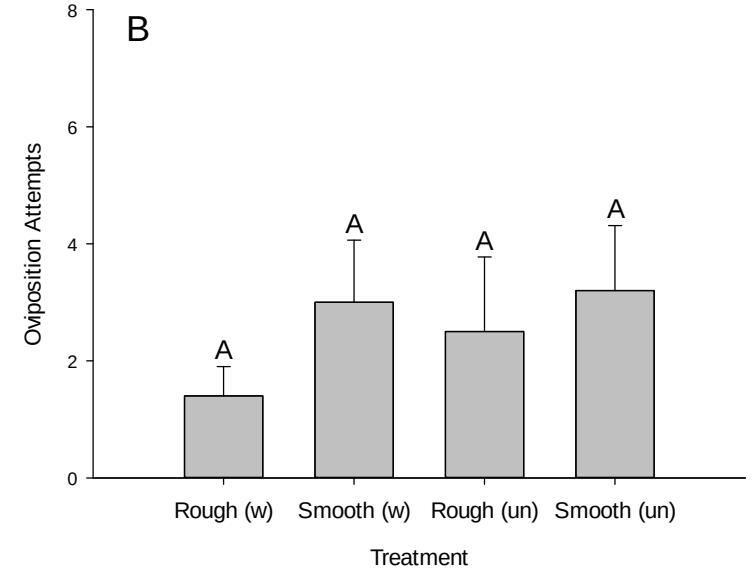

C
Successful Oviposition Attempts (%)
120
100
80
60
40
20
0
AB
A
B
Apple
Orange
Avocado
Passionfruit
Treatment

Figure 3.1 Mean (± S.E.) number of (A) visitations, (B) oviposition attempts and (C) percentage of successful oviposition events by *Bactrocera tryoni* into four fruits of differing peel properties under no-choice conditions. Columns surmounted with the same letter are not significantly different at $\alpha = 0.05$. A visitation is classified as an individual fly maintaining contact with the surface of the fruit for five seconds or more. An oviposition attempt is classified as the fly arching its abdomen approximately 60 - 70º with the fruit surface at the point of penetration and attempting to push its ovipositor through the peel. A successful oviposition event was classified as when the aculeus had penetrated the skin of the substrate and the ovipositor was held 90º in relation to the surface of the substrate. No successful oviposition events were recorded into passionfruit and it was not included in the cross-fruit analysis.


A
Visitations
14
12
10
8
6
4
2
0
A
A
A
A
Rough (w)
Smooth (w)
Rough (un)
Smooth (un)
Treatment


B
Oviposition Attempts
8
6
4
2
0
A
A
A
A
Rough (w)
Smooth (w)
Rough (un)
Smooth (un)
Treatment

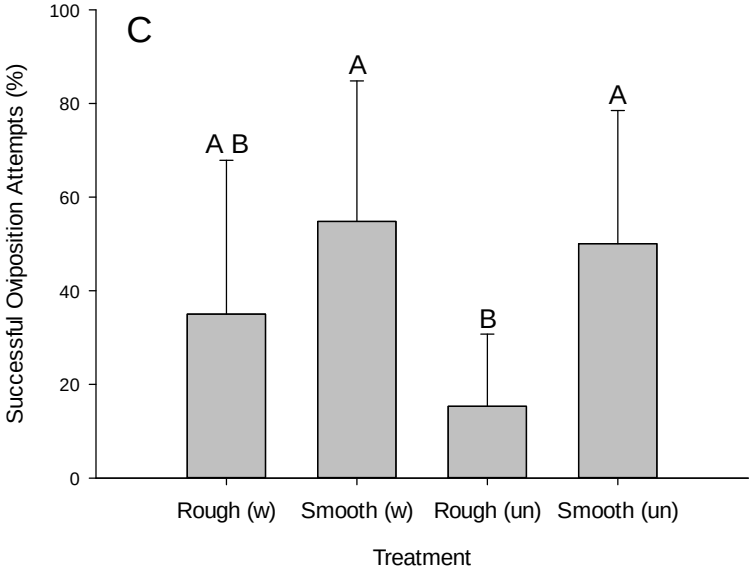
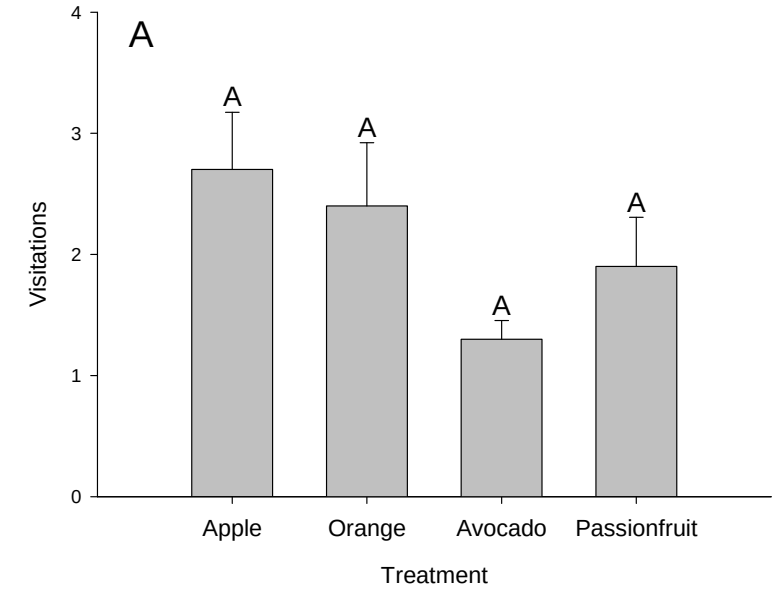
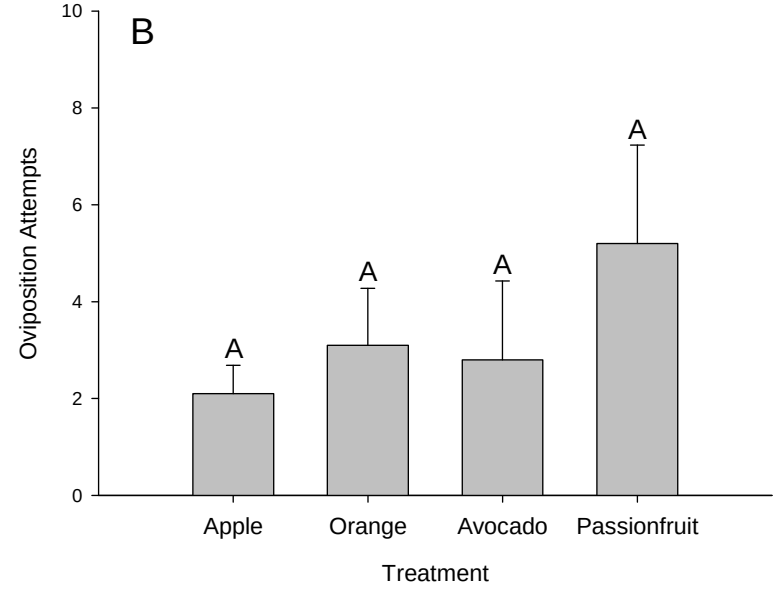

C
Successful Oviposition Attempts (%)
100
80
60
40
20
0
A B
A
B
A
Rough (w)
Smooth (w)
Rough (un)
Smooth (un)
Treatment

Figure 3.2 Mean (± S.E.) number of (A) visitations, (B) oviposition attempts and (C) percentage of successful oviposition events by *Bactrocera tryoni* into four agar fruit mimics of different surface type under no-choice condition. The agar mimics were simple agar gel (smooth), agar gel with vermiculite inclusions (rough), and were surface waxed (w) or unwaxed (un). Columns surmounted with the same letter are not significantly different at $\alpha = 0.05$. A visitation was classified as an individual fly maintaining contact with the surface of the fruit for 5 seconds or more. An oviposition attempt was classified as the fly arching its abdomen approximately 60 - 70º with the fruit surface at the point of penetration and attempting to push its ovipositor through the peel. A successful oviposition event was classified as when the aculeus had penetrated the skin of the substrate and the ovipositor was held 90º in relation to the surface of the substrate.

3.3.3 Experiment 2: Host preference and use in choice scenarios

Oviposition substrate did not have a significant impact upon the number of visitations, number of attempted of attempted oviposition events or the number of successful oviposition events for flies exposed to natural fruit hosts under choice conditions (respectively, $H = 6.236$, $P = 0.101$; $H = 0.889$, $P = 0.828$; $H = 5.805$, $P = 0.121$) (Fig. 3.3abc). This pattern was repeated when flies were exposed to agar fruit mimics, in which oviposition substrate did not have a significant impact upon the number of visitations, attempted oviposition events or the percentage of successful oviposition events (respectively, $H = 4.326$, $P = 0.228$; $H = 1.803$, $P = 0.614$; $F_{3,19} = 1.179$, $P = 0.344$) (Fig. 3.4abc). I recorded two examples of ovipunctures being reused within apples, and two examples of ovipuncture reuse for rough (unwaxed) fruit mimics.


A
Visitations
4
3
2
1
0
A
A
A
A
Apple
Orange
Avocado
Passionfruit
Treatment


B
Oviposition Attempts
10
8
6
4
2
0
A
A
A
A
Apple
Orange
Avocado
Passionfruit
Treatment

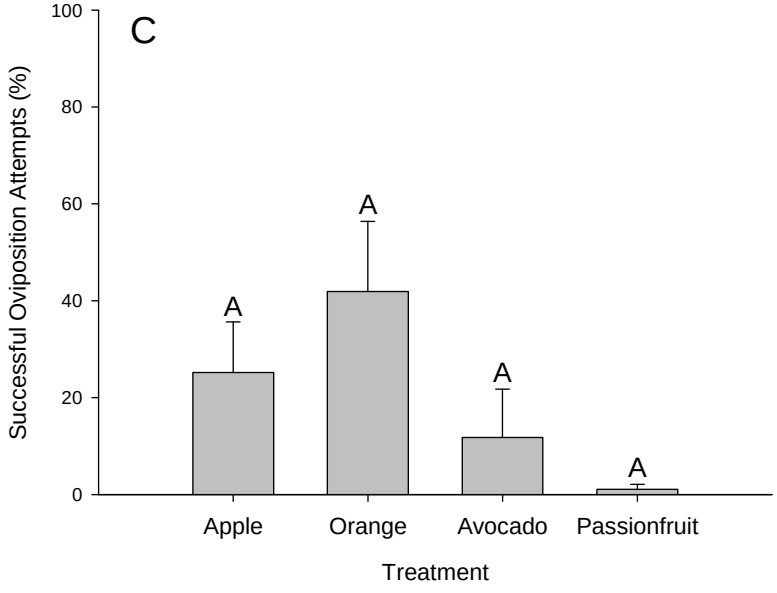
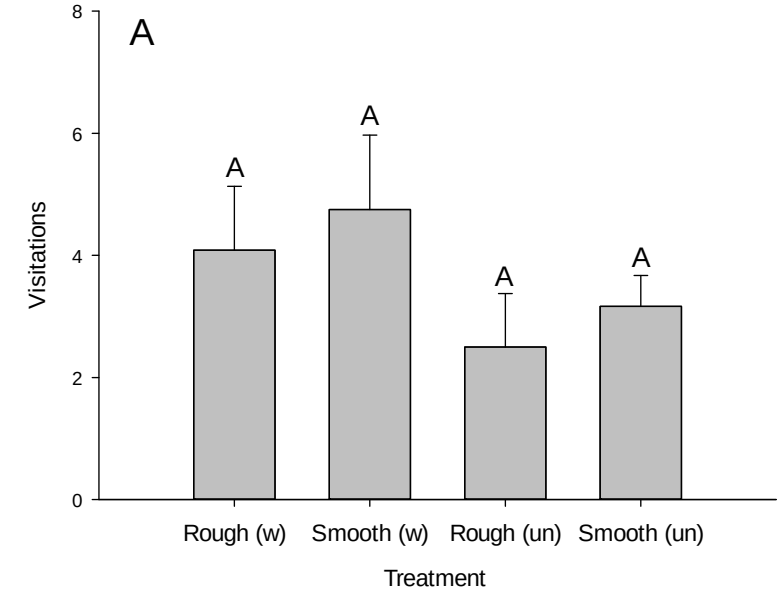
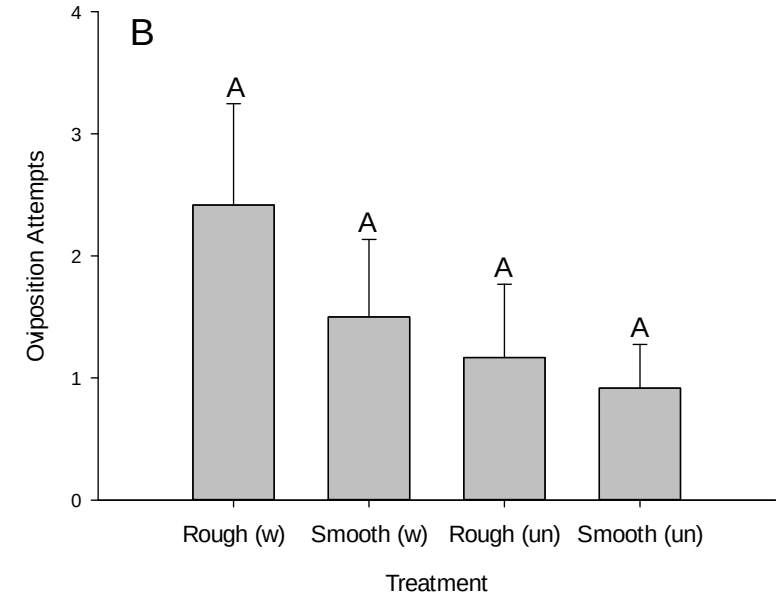

C
Successful Oviposition Attempts (%)
100
80
60
40
20
0
A
A
A
A
Apple
Orange
Avocado
Passionfruit
Treatment

Figure 3.3 Mean (± S.E.) number of (A) visitations, (B) oviposition attempts and (C) percentage of successful oviposition events by *Bactrocera tryoni* into four fruits of differing peel properties under choice conditions. Columns surmounted with the same letter are not significantly different at $\alpha = 0.05$. A visitation is classified as an individual fly maintaining contact with the surface of the fruit for five seconds or more. An oviposition attempt is classified as the fly arching its abdomen approximately 60 - 70º with the fruit surface at the point of penetration and attempting to push its ovipositor through the peel. A successful oviposition event was classified as when the aculeus had penetrated the skin of the substrate and the ovipositor was held 90º in relation to the surface of the substrate. No successful oviposition events were recorded into passionfruit and it was not included in the cross-fruit analysis.


A
Visitations
8
6
4
2
0
A
A
A
A
Rough (w)
Smooth (w)
Rough (un)
Smooth (un)
Treatment


B
Oviposition Attempts
4
3
2
1
0
A
A
A
A
Rough (w)
Smooth (w)
Rough (un)
Smooth (un)
Treatment

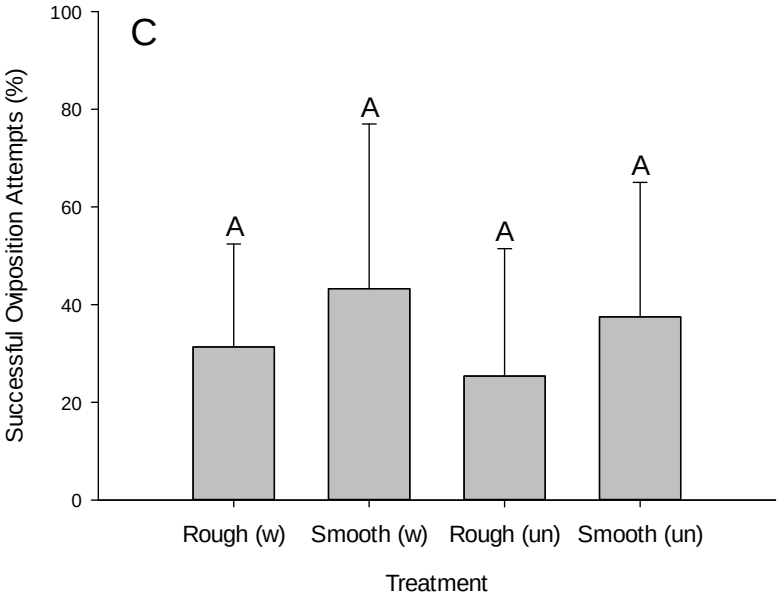

C
Successful Oviposition Attempts (%)
100
80
60
40
20
0
A
A
A
A
Rough (w)
Smooth (w)
Rough (un)
Smooth (un)
Treatment

Figure 3.4. Mean (± S.E.) number of (A) visitations, (B) oviposition attempts and (C) percentage of successful oviposition events by *Bactrocera tryoni* into four agar fruit mimics of different surface type under choice conditions. The agar mimics were simple agar gel (smooth), agar gel with vermiculite inclusions (rough), and were surface waxed (w) or unwaxed (un). Columns surmounted with the same letter are not significantly different at $\alpha = 0.05$. A visitation was classified as an individual fly maintaining contact with the surface of the fruit for 5 seconds or more. An oviposition attempt was classified as the fly arching its abdomen approximately 60 - 70º with the fruit surface at the point of penetration and attempting to push its ovipositor through the peel. A successful oviposition event was classified as when the aculeus had penetrated the skin of the substrate and the ovipositor was held 90º in relation to the surface of the substrate.

3.4 Discussion

The results from this study strongly suggest that *B. tryoni* does not discriminate for or against potential host fruit based upon their peel characteristics. There are no significant differences in the number of visitations or the number of attempted oviposition events for flies exposed to natural host fruit or agar fruit mimics during choice and no-choice experiments. This result was not entirely unanticipated, as results presented by Rattanpun *et al* (2009, 2010) demonstrated that the polyphagous fruit fly *B. dorsalis* was strongly attracted to different varieties of mango, regardless of peel toughness. Although passionfruit was almost entirely impenetrable, female *B. tryoni* did not discriminate against it in terms of visitations and oviposition attempts. This result emphasizes the fact that gravid female *B. tryoni* did not select against possible host fruit during the no-choice and choice trials based solely upon the qualities that contribute to overall peel penetration resistance. However, other traits than peel-toughness have been identified as factors influencing tephritid host selection and oviposition behaviours. These include host quality (e.g. size; colour), genetics (variability within and between populations), prior learning, number of ovarioles (potential fecundity), ovarian dynamics, aculeus wear, age, social context (e.g. facilitation; competition) and fruit volatiles (e.g. odour) (Aluja & Mangan, 2008).

During the no-choice observations orange received the greatest mean percentage of successful oviposition events, followed by apple and avocado. This result reflects the current consensus regarding peel toughness as a peel quality that limits oviposition. Although I did not detect any significant differences in successful oviposition events using natural fruit hosts during Experiment 2 (choice), the less powerful non-

parametric Kruskal-Wallis test may prevent the formal identification of a clear pattern of host-use. Figure 3.3c clearly shows that orange received the greatest mean number of successful oviposition events, followed by apple, avocado and passionfruit. This pattern of host-use is similar to that found in Experiment 1, other than the inclusion of passionfruit.

Fruit surface texture has also been identified as a potential factor influencing oviposition behaviour, although there is ambiguity regarding its impact (Diaz – Fleischer *et al*., 2001). Irregular fruit surfaces are more attractive to egg-laying insects such as the Diamondback moth *Plutella maculipennis* (Curtis), whereas exceptionally smooth or oily surfaces deter oviposition (Thorsteinson, 1960; Pritchard, 1969; Balagawi *et al*., 2005; Dhillon *et al*., 2005). A preference for irregular surfaces among tephritids is not universal, as female *B. oleae* (Rossi) attempted more ovipositions with smooth-surfaced fruit mimics rather than rough-surfaced mimics (Haniotakis & Voyadjoglou, 1978). The epicuticular wax layer often found on the surfaces of host fruit can either inhibit or assist oviposition, according to the species of fruit fly (Eigenbrode & Espelie, 1995; Eigenbrode, 2004). Results gathered during the no-choice observations indicated that *B. tryoni* preferred smooth-surfaced mimics with wax applied over rough-surfaced mimics without wax. Although my analyses did not reveal any statistically significant differences between host preference during choice observations, figure 3.4c shows that smooth-surfaced mimics with wax had the highest mean percentage of successful oviposition events in contrast to rough-surfaced mimics without wax, which had the lowest.

The results obtained from no-choice and choice trials were ambiguous, and may support multiple interpretations. I expected that *B. tryoni* females would rank host fruit according to their ability to penetrate their peel during no-choice and choice trials. Easily penetrated peel would be the most attractive to female fruit flies, whereas host fruit with impenetrable peel would be the least attractive. Likewise, I also anticipated that females would use unwaxed, rough-surfaced fruit mimics in preference to agar mimics with smooth surfaces and/or an application of wax. Instead, I observed a pattern of indiscriminate visitations between fruit hosts during no-choice and choice trials. Although I acknowledge that other well-known factors may have influenced host selection behaviour when considering the results for flies exposed to natural fruit hosts during no-choice and choice trials, my findings indicate that the different peel properties of the host fruit did not enhance or diminish their attractiveness.

The number of successful oviposition events in the no-choice trial favoured orange, which was the most easily penetrable. Furthermore, the fact that orange had the thickest peel of all the hosts used in the study implies that peel thickness does not play a significant role in host selection by *B. tryoni*. In contrast, I did not observe any significant difference in the number of successful oviposition events during choice observations. During no-choice trials *B. tryoni* females did not display a significant preference for any of the four types of agar fruit mimics presented to them. However, oviposition behaviour did appear to be influenced by surface properties, with more successful oviposition events occurring in agar mimics with smooth, waxed surfaces. No significant differences were observed during choice trials. The lack of significant differences in the number of visitations and attempted oviposition attempts for natural

hosts and agar mimics during no-choice and choice trials suggests that peel properties do not affect the attractiveness of host fruit to gravid *B. tryoni* females. The greater percentage of successful oviposition events recorded on orange hosts during no-choice trials would suggest that *B. tryoni* finds oviposition more easily accomplished in soft-peeled hosts. Additionally, the greater percentage of successful oviposition events using smooth-surfaced agar mimics covered with wax suggests that smooth peel and epicuticular wax do not restrict oviposition activity in *B. tryoni* females.

Chapter 4: Does larval host quality or peel penetrability best explain oviposition host choice in Queensland Fruit Fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae)

4.1 Introduction

Many ecological, behavioural and physiological components influence host plant preference in herbivorous insects, resulting in what has been described as a "dynamic hierarchy" (Via, 1990; Balagawi, 2005; Aluja & Mangan, 2008). A wide variety of hypotheses and models have been developed in order to explain the evolution of host range and observable host selection patterns by phytophagous insects. These models include, but are not restricted to the chemical coevolution hypothesis (Jermy, 1984; Bernays & Chapman, 1994); plant apparency and chemical defence hypothesis (Feeny, 1976); hierarchy threshold hypothesis (Courtney *et al*., 1989; Agnew & Singer, 2000); the enemy-free space hypothesis (Bernays & Graham, 1988); and optimality theory (Jaenike, 1978, 1990; Papaj, 1994). Optimality models feature prominently in theoretical and empirical research when exploring the host preference patterns of phytophagous insects, and include the widely used preference-performance model of host selection (Jaenike 1978, 1990; Papaj, 1994; Scheirs & De Bruyn, 2002; Scheirs *et al*., 2004).

The underlying assumption of the preference-performance model is that potential hosts are positively ranked in order of increasing suitability for larval development and survival (Jaenike, 1978; 1990; Thompson, 1988a; Papaj, 1994; Mayhew, 1997; Balagawi *et al*., 2013). This assumption has been violated in several studies, where phytophagous insects do not consistently choose hosts that are better for offspring survival (Santos *et al*., 2008; Gripenberg *et al*., 2010; Gillespie & Wratten, 2011). A review by Mayhew (1997) revealed a surprisingly large number of poor correlations between oviposition preference and host larval quality. Such 'bad motherhood' decisions have received considerable attention. Explanations include; optimal

foraging, where insects select seemingly inadequate larval hosts as a strategy to enhance their long-term fitness (i.e. longevity and egg production) at the expense of individual offspring (Mayhew, 2001; Scheirs 2002 & De Bruyn; Uesugi, 2009); an attempt to access enemy-free space (Grewal & Kapor, 1986; Mangel *et al*., 1994; Purcell *et al*., 1994; Yuval & Hendrichs, 2000; Diaz-Fleischer & Aluja, 2003c); limited neural capacity, where polyphagous insects make poorer decisions compared to specialised insects due to their inability to effectively process stimuli from different hosts (Bernays, 2001; Aluja & Mangan, 2008; Gripenberg *et al*., 2010); or a reduction of the insect's preference thresholds if they do not have access to a preferred host while under a heavy egg load (Fitt, 1986a).

Polyphagous tephritid fruit flies (Diptera: Tephritidae) are one herbivore group for which poor correlations between adult choice and offspring performance have been recorded. While most of the above explanations have been applied to tephritids (Fitt, 1986a; Rattanapun *et al*., 2009; Balagawi *et al*., 2013), there is another possible reason which relates explicitly to the mechanics of fruit use by fruit flies. Frugivorous fruit flies lay their eggs into, or through the peel of fruit which then become feeding hosts for their larvae (Bateman, 1972). It is thought that the host range of tephritid fruit flies may be restricted by the mechanical ability of the fly to penetrate fruit peel with their ovipositor, although this assumption has not been tested (Aluja *et al*., 2004; Balagawi *et al*., 2005; Rattanapun *et al*., 2009; 2010). Although not stated directly, there is also a clear inference in the literature that a blunted ovipositor may reduce oviposition opportunities and host range (Jones & Kim, 1994; Diaz-Fleischer *et al*., 2001; Aluja & Mangan, 2008), although only Jones & Kim (1994) have explicitly identified abraded ovipositors in tephritids (from four species; *Rhagoletis pomonella*

(Walsh), *R. Mendax* (Curran), *Ceratitis capitata* and *Bactrocera oleae* (Gmelin)) and no authors have looked to see if this does modify host range.

While it has been demonstrated for several tephritid species that an increase in peel toughness will limit a fly's ability to penetrate peel and lay eggs (Messina *et al*., 1991; Balagawi *et al*., 2005; Dhillon *et al*., 2005) - and that a positive relationship exists between oviposition preference and peel penetrability (Sharma & Amritphale, 2008), there is another body of literature that contradicts the generality of such findings. Papachristos & Papadopoulos (2009) found for *C. capitata* that an increase in peel toughness was not associated with a reduction in oviposition. There is also speculation that a firm peel may be regarded favourably by ovipositing flies and used as a measure of host quality because of the close association between fruit firmness and the maturity of the host (Greany *et al*., 1985; Messina & Jones, 1990; Diaz-Fleischer & Aluja, 2003b).

Using a polyphagous fruit fly, *B. tryoni*, the purpose of this study is to test the assumption that the fly's realised host range differs from its potential host range because of mechanical restrictions imposed by the potential host fruit, and that a potentially positive adult preference/offspring performance relationship is by this disrupted. Specifically, I observed whether gravid female *B. tryoni* selected host fruit of high offspring suitability, but with tough peel, or host fruit of poor offspring suitability but with easily penetrable peel. If the flies are most strongly influenced by selection to maximise larval survival, I predict that ovipositing females will select high quality hosts despite their mechanically resistant peel. Alternatively, should host selection be driven by selection to maximise adult performance, I predict that females

will exhibit a preference towards host plants that have soft peel independent of offspring performance.

4.2 Materials & Methods

4.2.1 Overview of Experiments

Choice and no-choice experiments were used to assess *B. tryoni* host preference with respect to two factors: the ability of host peel to resist oviposition and the suitability of the host for offspring development. Four fruit species were used in trials, two of which were hard-peeled (i.e. poor oviposition hosts) but which were good larval hosts (i.e. high offspring survival) and two of which were soft-peeled but poor larval hosts. In the no-choice trials (Experiment 1) female flies were offered only a single fruit species, while in the choice trials (Experiment 2) all four fruit types were offered simultaneously.

4.2.2 Common Methods

Laboratory flies

All flies used in laboratory studies were obtained from cultures maintained by the Queensland Governments Department of Agriculture, Fisheries and Forestry (QDAFF), Boggo Road Ecosciences Precinct, Brisbane. The cultures were up to 34 generations old, refreshed every two generations with wild material and reared on carrot-based medium (Christenson *et al*., 1956). For use in experiments, pupae were received from QDAFF and the emergent adults held under ambient conditions at the Queensland University of Technology. Adults held during experimental trials had *ad*

libitum access to sugar, hydrolysed yeast (MP Biomedicals Australasia Pty. Ltd.) and water.

Fruit

Four fruit types were used in experiments: mango (*Mangifera indica* Kensington pride), papaya (*Carica papaya* commercially grown yellow), rockmelon (*Cucumis melo* Sweet) and passionfruit (*Passiflora edulis* Panama red). Each fruit type is regarded as a host or major host for *B. tryoni* (Hancock *et al*., 2000). The selection of these four fruit types followed a preliminary study of 11 fruit species to identify fruit possessing the desired experimental combinations of hard peel but good offspring performance, and soft peel but poor offspring performance. All fruit used were purchased as organic from a commercial supplier and any fruit whose peel displayed any signs of damage was rejected.

Fruit peel properties

Ten randomly chosen ripe pieces for each fruit type were assessed for peel penetration resistance and elasticity with the results used to interpret patterns of host-use. Penetration resistance measurements were made using a penetrometer (QA Supplies Model FT 327 Fruit Pressure Tester, QA Supplies, Norfolk, USA), which measured the force (N) required to penetrate a fruit using a 1 mm diameter probe in a period of two seconds. Three puncture tests per fruit were performed. Elasticity was measured through calculations of the fruit's toughness index (TI) using the average of the three readings for each fruit with the formula: TI = average force for 2 seconds / (number of peel penetrations out of three attempts + 1).

Larval Survival Trials

In order to assess host fruit suitability for larval survival, trials were conducted in which host fruit were artificially infested with *B. tryoni* eggs. The eggs were collected from hollowed-out apple domes and washed using de-ionized water. For each type of host fruit six individual specimens had two small sections of their peel removed, 15 eggs were placed inside of each peel opening (i.e. 30 eggs per fruit piece) and the removed peel section was replaced over the eggs. The fruit were placed on separate trays containing vermiculite, and after ten days the vermiculite was sieved for pupae.

Behavioural observations

Behavioural studies were performed in three, two hour observation blocks between 0800 – 1400 hr. All observations were conducted outdoors natural temperature and lighting conditions between December 2011 and February 2012. Additional observations were performed between August and September 2012. Observations commenced with the introduction of three gravid, ovipositionally-naive female flies into a clear Perspex cage (40x40x40 cm) containing host fruit. Four cages were used in each observation block.

Host fruit preference was scored as: (i) the number of fly visitations to a fruit; (ii) the number of attempted oviposition events; (iii) the proportion of oviposition attempts that were successful; and (iv) the number of times that previously made ovipunctures were reused. A visitation was defined as an individual fly maintaining contact with the surface of the fruit for five seconds or more. An oviposition attempt was defined as the fly arching its abdomen approximately 60 - 70º with the fruit surface at the point of penetration and attempting to push its ovipositor through the peel. A successful oviposition event was defined as when the ovipositor had clearly penetrated the skin of the fruit and it brought to an angle of 90º with the fruit surface

(Pritchard, 1969). At the conclusion of each block of observations both flies and host fruit were replaced with fresh specimens. If no activity was witnessed inside a cage after 20 minutes both the flies and fruit were replaced with fresh specimens.

For no-choice trials 12 replicates were made of three flies exposed to a single fruit piece for two hours. For choice trials the same replication was used, except individual pieces of all four fruit types were offered simultaneously. Position of fruit types within an observation cage were randomised between replicates. The point of stem attachment of each fruit was covered by a piece of adhesive paper to prevent flies from exploiting this naturally weak point of the fruit, which would not be naturally exposed if fruit were still on the plants.

4.2.3 Data analysis

The treatment means (separately for the choice and no-choice trials) were compared through one-way analysis of variance (1-way ANOVA) following tests for normality (Levene's & Kolmogorov-Smirnov tests) and subsequent Log10 transformations if required. Where data could not be normalised, the nonparametric Kruskal-Wallis test was used as an alternative. Tukey's test and the Game-Howell test were the post-hoc tests used, if required, for the ANOVA and Kruskal-Wallis tests, respectively. All tests were conducted with a confidence interval of 95% and results are presented as the mean ± 1 S.E.

4.3 Results

4.3.1 Fruit quality trials

There were significant differences between the four fruit types in the number of pupae produced (H = 12.576; P = 0.006). Mango proved to be the worst host, followed by papaya. Rockmelon was a significantly better host than mango, while passionfruit produced the greatest mean number of pupae. (Fig. 4.1).

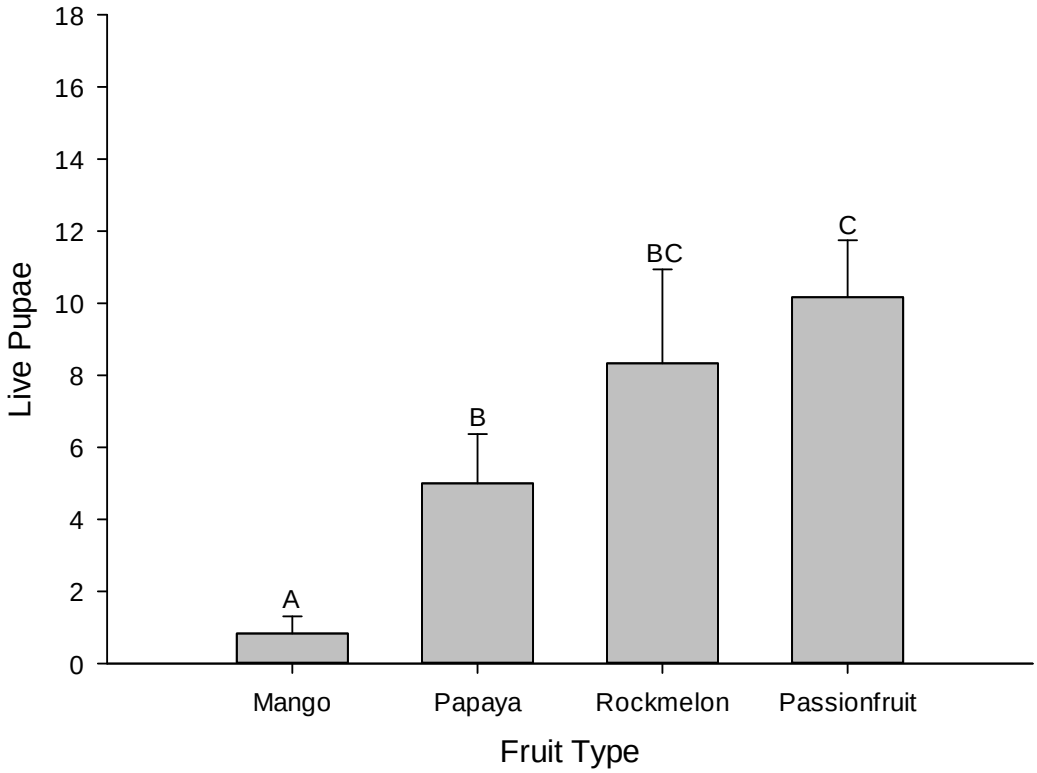


Figure 4.1 Mean (± S.E.) number of *Bactrocera tryoni* pupae recovered from four fruit types. Thirty eggs were initially placed into each of three fruit pieces per fruit type.

4.3.2 Peel properties

There was a significant difference in peel penetration resistance (N) ($H = 98.022$; $P < 0.001$) and peel elasticity ($F_{3,36} = 162.867$; $P < 0.001$) between the four fruit types (Fig. 4.2ab). Mango was intermediate in both peel attributes between rockmelon/passionfruit and papaya, while papaya had the lowest scores for both attributes. Both mango and papaya were significantly different in both traits to all other fruit types. Rockmelon and passionfruit scored the highest for peel penetration resistance and peel elasticity values, but they were not significantly different from each other.

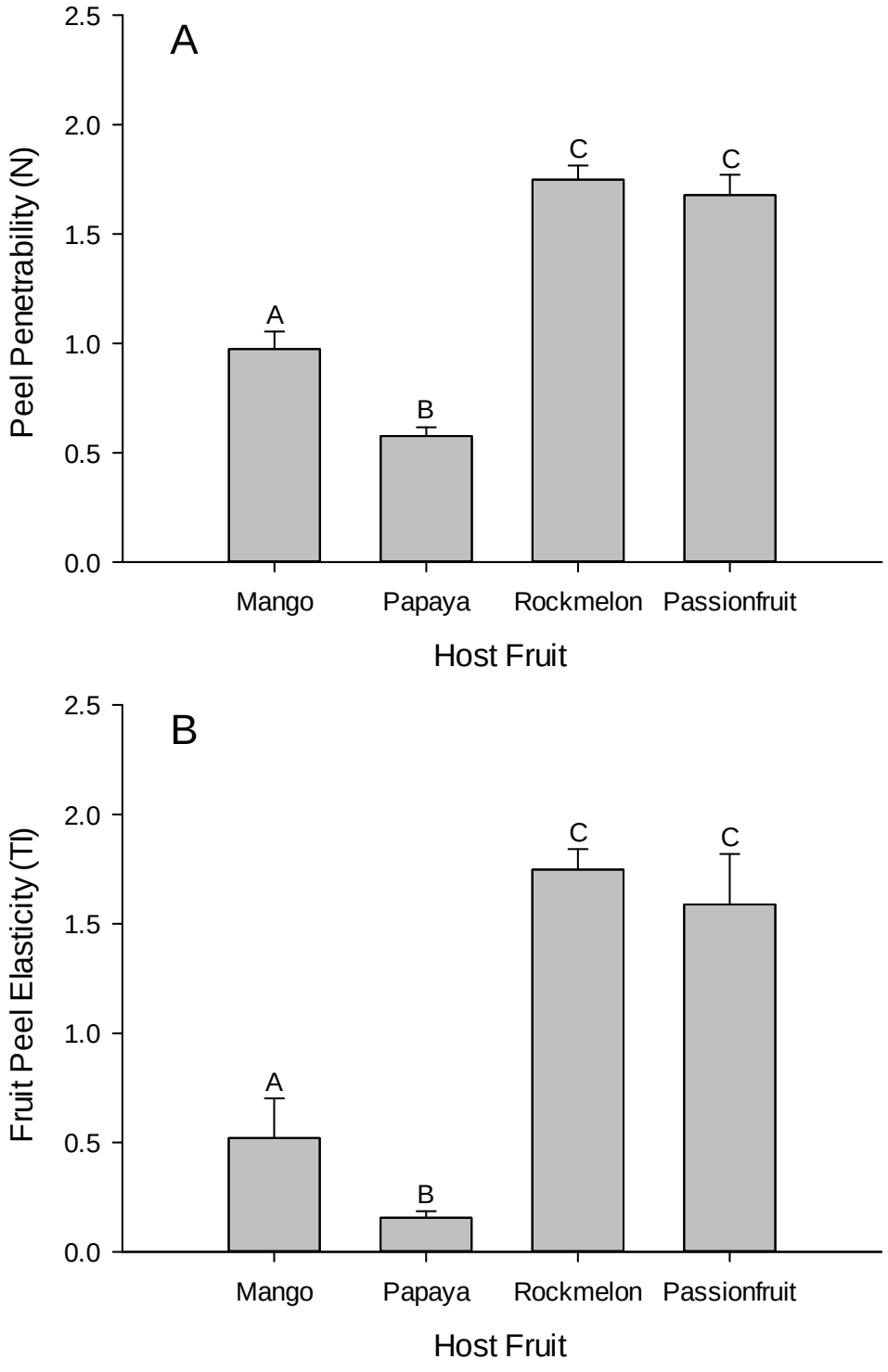


Figure 4.2 Mean (± S.E.) measurement of (A) host peel penetrability resistance and; (B) host peel elasticity. Columns surmounted with the same letter are not significantly different at $\alpha = 0.05$.

4.3.3 Experiment 1: Host preference and use in no-choice scenarios

Significant differences were found in the mean number of visitations ($H = 12.537$; $P = 0.006$) and oviposition attempts ($H = 7.916$; $P = 0.048$) by female *B. tryoni* to the four fruit types (Fig. 4.3ab). Post-hoc analysis revealed that the mean number of visitations to passionfruit was significantly greater than to papaya and rockmelon, with mango intermediate (Fig. 4.3a). Having arrived at a fruit, the mean number of oviposition attempts into passionfruit, rockmelon and mango were not significantly different to each other. Papaya received significantly fewer oviposition attempts than other fruit, with the exception of mango (Fig. 4.3b). No successful oviposition occurred into passionfruit in any replicate. For the three remaining fruit, no significant differences were found in the mean number ($H = 1.315$; $P = 0.518$, Fig. 4.3c) or mean percentage of successful oviposition ($H = 1.400$; $P = 0.497$, Fig. 4.3d) attempts by female *B. tryoni*.

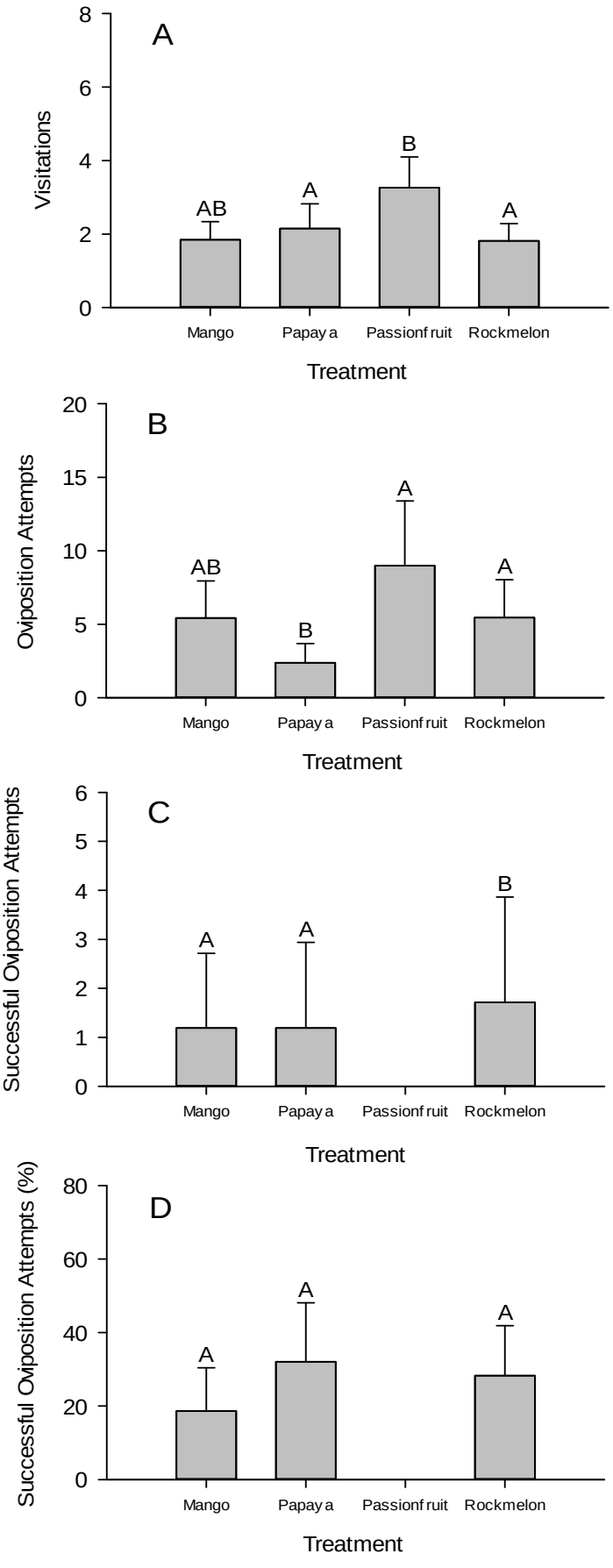

A
Visitations
8
6
4
2
0
AB
A
B
A
Mango
Papaya
Passionfruit
Rockmelon
Treatment
B
Oviposition Attempts
20
15
10
5
0
AB
B
A
A
Mango
Papaya
Passionfruit
Rockmelon
Treatment
C
Successful Oviposition Attempts
6
5
4
3
2
1
0
A
A
B
Mango
Papaya
Passionfruit
Rockmelon
Treatment
D
Successful Oviposition Attempts (%)
80
60
40
20
0
A
A
A
Mango
Papaya
Passionfruit
Rockmelon
Treatment

Figure 4.3 Mean (± S.E.) number of (A) visitations, (B) oviposition attempts, (C) the mean number of successful oviposition attempts; and (D) the mean percentage of successful oviposition attempts by *Bactrocera tryoni* into fruit hosts of different surface hardness and larval suitability during no-choice experiments. Mango and papaya were soft-skinned hosts of poor suitability for *B. tryoni* larvae, while passionfruit and rockmelon were hard-skinned hosts well suited for *B. tryoni* larvae. Columns surmounted with the same letter are not significantly different at $\alpha = 0.05$. A visitation was classified as an individual fly maintaining contact with the surface of the fruit for 5 seconds or more. An oviposition attempt was classified as the fly arching its abdomen approximately 60 - 70º with the fruit surface at the point of penetration and attempting to push its ovipositor through the peel. A successful oviposition event was classified as when the aculeus had penetrated the skin of the substrate and the ovipositor was held 90º in relation to the surface of the substrate.

4.3.4 Experiment 2: Host preference and use in choice scenarios

Significant differences were found in the mean number of visitations ($H = 11.271$; $P = 0.010$), percentage of successful oviposition attempts ($H = 23.503$; $P < 0.001$) and the mean number of successful oviposition ($H = 0.007$; $P = 0.007$) attempts by female *B. tryoni* (Fig. 4.4acd). Post-hoc analysis revealed that the number of visitations to rockmelon was significantly greater than to mango and passionfruit, with papaya intermediate (Fig. 4.4a). Having arrived at the fruit, no significant difference was detected in the number of oviposition attempts between different hosts ($H = 4.850$; $P = 0.183$) (Figure 4.3b). After commencing oviposition, the mean percentage of successful oviposition attempts into papaya was significantly greater than those witnessed for mango and passionfruit, with rockmelon intermediate (Fig. 4.3d). The number of successful oviposition attempts into passionfruit was significantly lower in comparison to mango, papaya and rockmelon (Fig. 4.3c).

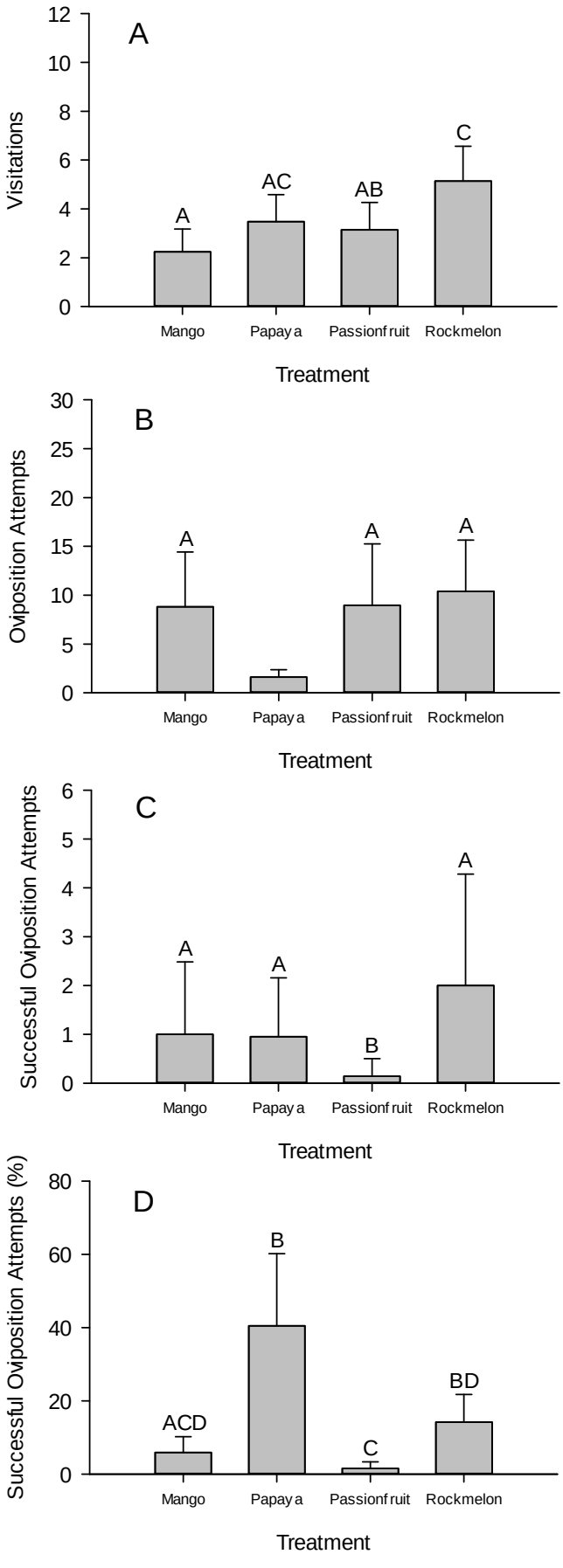

A
Visitations
12
10
8
6
4
2
0
A
AC
AB
C
Mango
Papaya
Passionfruit
Rockmelon
Treatment
B
Oviposition Attempts
30
25
20
15
10
5
0
A
A
A
Mango
Papaya
Passionfruit
Rockmelon
Treatment
C
Successful Oviposition Attempts
6
5
4
3
2
1
0
A
A
B
A
Mango
Papaya
Passionfruit
Rockmelon
Treatment
D
Successful Oviposition Attempts (%)
80
60
40
20
0
ACD
B
C
BD
Mango
Papaya
Passionfruit
Rockmelon
Treatment

Figure 4.4 Mean (± S.E.) number of (A) visitations, (B) oviposition attempts, (C) the mean number of successful oviposition attempts; and (D) the mean percentage of successful oviposition attempts by *Bactrocera tryoni* into fruit hosts of different surface hardness and larval suitability during choice experiments. Mango and papaya were soft-skinned hosts of poor suitability for *B. tryoni* larvae, while passionfruit and rockmelon were hard-skinned hosts well suited for *B. tryoni* larvae. Columns surmounted with the same letter are not significantly different at $\alpha = 0.05$. A visitation was classified as an individual fly maintaining contact with the surface of the fruit for 5 seconds or more. An oviposition attempt was classified as the fly arching its abdomen approximately 60 - 70º with the fruit surface at the point of penetration and attempting to push its ovipositor through the peel. A successful oviposition event was classified as when the aculeus had penetrated the skin of the substrate and the ovipositor was held 90º in relation to the surface of the substrate.

4.4 Discussion

I observed in Experiment 1 that passionfruit received a significantly greater number of visitations in comparison to rockmelon and papaya. Although mango received the least number of visitations, it was not significantly different compared to passionfruit. Consequently, I consider that female *B. tryoni* perceived passionfruit as the most attractive of the four hosts. A clear pattern was more difficult to discern when I assessed oviposition attempts. Significantly fewer oviposition attempts were recorded for papaya when compared to passionfruit and rockmelon. Passionfruit received the greatest number of oviposition attempts, although it was not significantly different when compared against mango and rockmelon. There was no significant difference between mango, papaya and rockmelon for the percentage of successful oviposition attempts.

Similar patterns presented themselves when I assessed the results for Experiment 2. Rockmelon received a significantly greater number of visitations in comparison to passionfruit and mango, leading us to conclude that it was the most attractive host-used during this experiment. As with Experiment 1, I was confronted with an ambiguous pattern when assessing oviposition attempts for Experiment 2. Although rockmelon received significantly more oviposition attempts when compared against papaya, it was not significantly different compared against mango or passionfruit. Furthermore, the number of oviposition attempts for papaya was not significantly different compared against mango or passionfruit. However, papaya's status as the most easily penetrable host was reflected by it having a significantly greater percentage of successful oviposition attempts when compared against mango and passionfruit. The results suggest that female fruit flies did not select against hosts

based upon the ability of their peels to resist oviposition, and such a result could be used as support for the preference-performance hypothesis. The resistant peels of passionfruit and rockmelon may have been interpreted as an indicator of high quality (Greany *et al*., 1985; Messina & Jones, 1990; Diaz-Fleischer & Aluja, 2003b).

Ovipositor wear has been found in four species of tephritid fruit flies (Jones & Kim, 1994). There is a strong inference from multiple sources that increased ovipositor wear from using hard-peeled hosts will reduce oviposition opportunities and host range (Jones & Kim, 1994; Diaz-Fleischer *et al*., 2001; Aluja & Mangan, 2008). Progressive cuticular wear believed to have been brought about by substratum hardness and age has been identified in the mandibles of phytophagous insects, and such wear are believed to negatively affect feeding efficiency (Arens, 1990; Wallin, 1988; Kohler *et al*., 2000; Roitberg *et al*., 2005; Vincent, 2009). I predicted that flies would display a preference for soft-peeled hosts in order to reduce potential ovipositor wear. The continued use of passionfruit and rockmelon lead me to conclude that gravid *B. tryoni* females did not select against oviposition-resistant hosts. Although this outcome could be interpreted as supporting the preference-performance model, the results obtained for mango introduces a degree of uncertainty. If phytophagous insects rank positively rank hosts according to larval suitability, it is reasonable to assume that mango would be the least preferred host. This prediction was not borne out in Experiment 1, where no significant differences were detected for the number of visitations or oviposition attempts between mango and the three remaining hosts. The number of visitations made to mango in Experiment 2 was only significantly different when compared against rockmelon.

The use of unsuitable larval hosts by phytophagous insects is not unprecedented (Santos *et al*., 2008; Gripenberg *et al*., 2010; Gillespie & Wratten, 2011) and has been attributed to a number of different reasons including; (1) maximise individual fitness at the expense of offspring; (2) allow larvae to evade predation; (3) neural constraints; (4) or a reduction of the preference threshold (Grewal & Kapor, 1986; Yuval & Henrichs, 2000; Aluja & Mangan, 2008; Gripenberg *et al*., 2010; Clarke *et al*., 2011). Furthermore, host selection is not a simple process and is the result of the interaction between a wide variety of ecological, behavioural and physiological components (Via, 1990; Balagawi, 2005; Aluja & Mangan, 2008). Finally, it should be kept in mind that adaptations of the optimal oviposition theory including the preference-performance hypothesis are not intended to accurately predict potential host-animal associations, and are intended to assist us interpreting observed patterns of host-use (Owens, 2006).

The wide variety of hosts used in this study opens the possibility of unforseen confounding effects influencing the observed host preferences and oviposition behaviours of *B. tryoni*. It is widely accepted that visual and olfactory cues play key roles in host selection and oviposition of dacine fruit flies, and I cannot discount the possibility that such factors may have influenced fruit fly behaviour (Bateman, 1972; Finch & Collier, 2000; Pinero *et al*., 2006; Aluja & Mangan, 2008). Nevertheless, this risk was unavoidable given that the goal of this study was to identify a link between host-use restriction brought about by peel resistance to oviposition and a broader reduction in potential host range. Based upon current results, I cannot definitively state that fruit flies selected for or against hosts based upon larval quality. Although the poor larval quality papaya was the least acceptable host based on the number of oviposition attempts, I did not record any significant differences in the number of

oviposition attempts between hard-peeled hosts and mango, which was the poorest quality host. However, the high number of visitations and oviposition attempts recorded for hard-peeled hosts clearly demonstrates that the potential host range of *B. tryoni* is not limited by peel-resistance to oviposition.

Chapter 5: Testing for the Presence of Hardness-Linked Transition Elements within the Aculei of Three Species of Tephritid Fruit Fly

5.1 Introduction

This thesis was built on an assumption that the aculeus of *B. tryoni* would wear and that this would influence host range (Kim & Jones, 1994; Aluja *et al*., 2004; Balagawi *et al*., 2005; Rattanapun *et al*., 2009; 2010). Certainly, Kim and Jones (1994) demonstrated aculeus wear in *Ceratitis* and *Rhagoletis* spp., and in other systems cuticular wear of structures such as mandibles have been demonstrated and shown to result in a loss of structure efficiency (Arens, 1990; Wallin, 1988; Kohler *et al*., 2000; Roitberg *et al*., 2005; Vincent, 2009). Based on such studies, my initial hypotheses were that aculeus wear would occur and act as a limiting factor upon the realised host range of *B. tryoni*.

However, the aculei of *B. tryoni* displayed few signs of wear in Chapter 2, while host preference experiments (Chapters 3 and 4) suggest that females do not select against hosts with tough peel. If the use of hard-peeled hosts by *B. tryoni* females results in little aculeus wear, I believe that the next logical step is to explore physiological and behavioural mechanisms linked to wear resistance in arthropod cuticle. The relative lack of wear among aculei belonging to *B. tryoni* provided a stark contrast to the heavily worn *C. capitata* aculei observed by Jones & Kim (1994). This led me to speculate that differences in elemental composition of the cuticle could account for the different patterns of aculeus wear.

The external cuticle of insects is a low-weight, composite material consisting of a procuticle composed of chitin filaments arranged within a protein matrix and covered by an epicuticle consisting of lipids and proteins (Vincent & Wegst, 2004; Andersen, 2010). Cuticle provides the insect structural support and protection from the

environment, while remaining light enough to allow flight (Andersen, 2010; Vilhelmsen & Turrisi, 2011). Insect cuticles are diverse materials, which vary in thickness, stiffness, strength and colour (Andersen, 2010). The structural properties of insect cuticle can range from soft and flexible to strong and hard, depending on their functional role (Cribb *et al*., 2010; Vilhelmsen & Turrisi, 2011). Soft and flexible cuticle can be found in joints, whereas the cuticle of 'tools' such as mandibles, claws and ovipositors that experience significant interaction with high friction materials are hard, elastically stiff, tough and possess high abrasion resistance (Schoberl & Jager, 2006; Cribb *et al*., 2010).

Numerous authors have linked the presence of inorganic materials such as zinc (Zn) to increased wear resistance in the cuticles of grasshoppers and locusts (Orthoptera) and ants (Hymenoptera) (Hillerton & Vincent, 1982; Hillerton *et al*., 1982; Edwards *et al*., 1993; Quicke *et al*., 1998; Schofield *et al*., 2002; 2003; Vincent & Wegst, 2004). The concentration of transition metals, including Zn and manganese (Mn), in the cuticle of mandibles, mouth hooks, claws and ovipositors has been perceived as an evolutionary mechanism to minimise wear in these structures. When present the metals are concentrated in specific locations within a structure, such as the cutting edges of mandibles (Hillerton & Vincent, 1982; Hillerton *et* al., 1984; Fontaine *et al*., 1991; Quicke *et al*., 1998; Schofield, 2001; Morgan *et al*., 2003; Schofield, 2005; Cribb *et al*., 2008a). The inclusion of heavy elements within the cuticle occurs during sclerotization (= tanning), when cross-bonding of the protein molecules happens and the cuticle typically darkens in colour (Cribb *et al*., 2010). The amount of transition metal witnessed in cases of cuticlar hardening can reach upwards of 16% of dry mass (Schofield *et al*., 2002; Morgan *et al*., 2003; Cribb *et al*., 2008b).

Zn has often been identified as a hardening agent of insect cuticle, and tests have demonstrated that Zn enriched cuticle is often harder compared to unenriched cuticle (Schofield *et al*., 2002; 2003; Broomell *et al*., 2008; Cribb *et al*., 2008ab). In contrast, Mn enriched cuticle is often less hard, leading to speculation that its inclusion serves different purposes, including increased density, tensile strength, compressive strength and fracture resistance (Quicke *et al*., 1998; Morgan *et al*., 2003; Cribb *et al*., 2010). Only a single study examining the jaw of a marine annelid, *Nereis virens,* has positively linked the addition of Mn to increased hardness (Broomell *et al*., 2008).

Quicke *et al*. (1998) assessed the elemental makeup of aculei from various species of wasps and found that the aculei of species which had to penetrate hard substrates were typically enriched with transition metals, whereas the aculei of wasps that did not penetrate hard substrates to oviposit were often unenriched. This led me to speculate that maybe the reason that Jones & Kim (1994) found wear in the aculei of *C. capitata*, while I found (at most) only minor wear in the aculei of *B. tryoni*, was because of difference in cuticular metal inclusions between the two species. The purpose of this experiment was thus to identify if there were differences in the elemental composition of aculei taken from laboratory-bred specimens of Queensland fruit fly and the Mediterranean fruit fly (*C. capitata*), to see if this might correlate with the apparent wear resistant nature of *B. tryoni* aculei. As an addition to the experiment, I also studied the olive fruit fly (*B. oleae* Rossi), as a congeneric species with *B. tryoni*, but a monophagoues (on olive) fruit fly rather than very highly polyphagous Queensland and Mediterranean fruit flies (Hancock *et al*., 2000; Wharton *et al*., 2000; Clarke *et al*., 2011; Navarro-Llopis *et al*., 2011; Karsten *et al*.,

2013). Energy dispersive X-ray microanalysis was used to compare the elemental composition of aculei obtained from the three fruit fly species.

5.2 Materials & Methods

5.2.1 Sample Collection and Preparation

Bactrocera tryoni were obtained from cultures maintained by the Queensland Government Department of Agriculture, Fisheries and Forestry (QDAFF), Boggo Road Ecosciences Precinct, Brisbane. The cultures were up to 34 generations old, refreshed every two generations with wild material and reared on carrot-based medium. Mediterranean fruit flies were obtained from cultures maintained by the Western Australian Government Department of Agriculture and Food. The *C. capitata* cultures from which specimens were obtained were refreshed with wild material approximately one month before I received the specimens. Olive fruit flies were provided by the Insect Pest Control Laboratory, Joint FAO/IAEA Agriculture and Biotechnology Laboratories, A-2444 Seibersdorf, Austria. All specimens were euthanized one week after emergence, to allow development of the exocuticle (Evans, 1967). The excised aculei were air dried for seven days at room temperature prior to examination.

5.2.2 X-ray Microanalysis

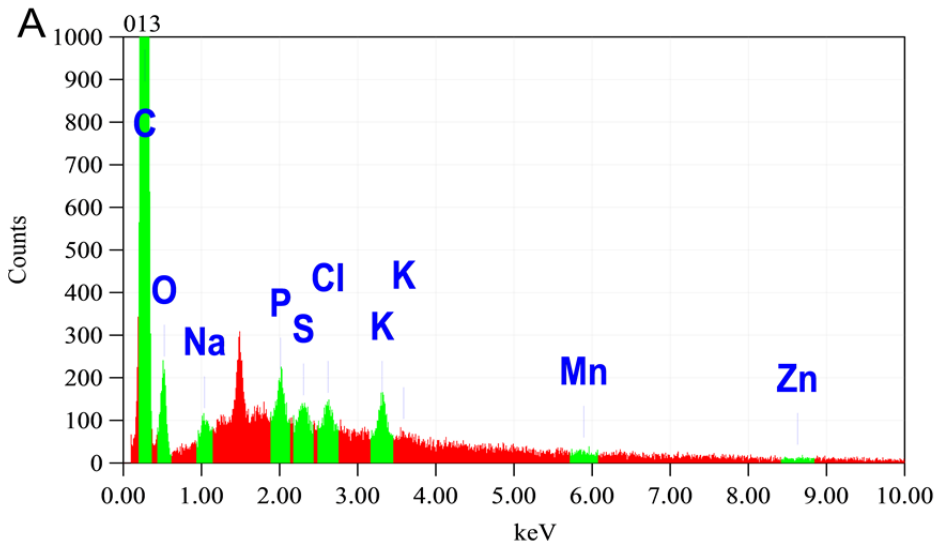
Aculei samples were assessed for elemental composition using energy dispersive X-ray analysis. Aculeus specimens were analysed using a Jeol JSM-6460 (LA) scanning electron microscope maintained by the University of Queensland, Brisbane. Analysis

of the unembedded aculeus specimens was performed in low-vacuum mode, with standardless quantitiation accomplished using the JED-2300 energy dispersive X-ray analyser. Acquisition conditions for scanning electron microscopy (SEM) were 20 keV, 10 mm working distance and 40 s live time acquisition. The lack of a suitable standard to compare samples against necessitated the use of standardless quantitiation with a PRZ correction. Quantification is reported generically against values as trace (<0.5 mass% dry weight), minor (a few percent) or major (>5% to ≥ 20% mass weight) as outlined by Newbury (1991). Ten aculei taken from *B. tryoni* were examined, along with three aculei each taken from *B. oleae* and *C. capitata*. The beam was directed at the aculeus apex, where I expected to find measureable quantities of transition metals.

Although I originally intended to use nanoindentation testing to assess the hardness and elasticity of fruit fly aculei, technical challenges and time limitations forced the removal of this component from the study.

5.3 Results

X-ray microanalysis of aculeus specimens taken from *B. tryoni*, *B. oleae* and *C. capitata* did not find Zn or Mn in measureable quantities. The representative spectra of adult fruit fly aculei are presented in Figure 5.1.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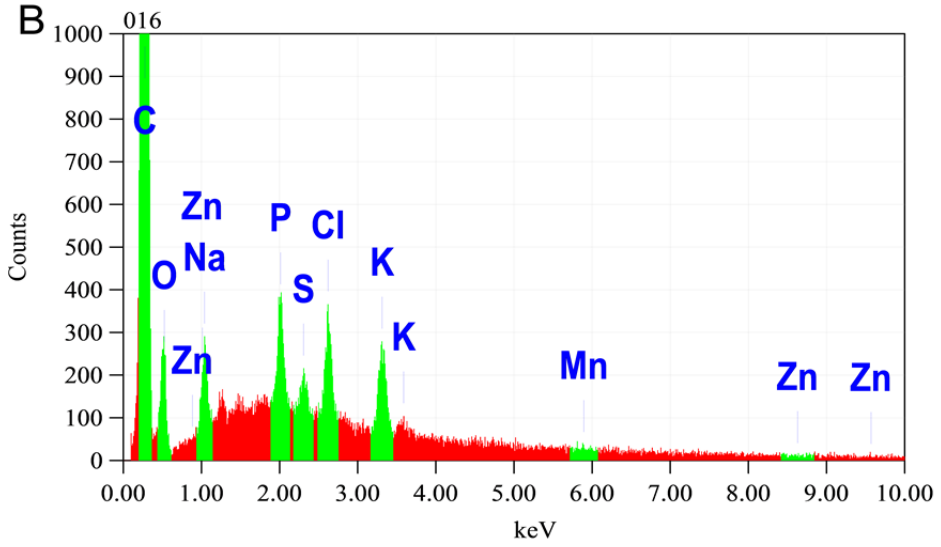
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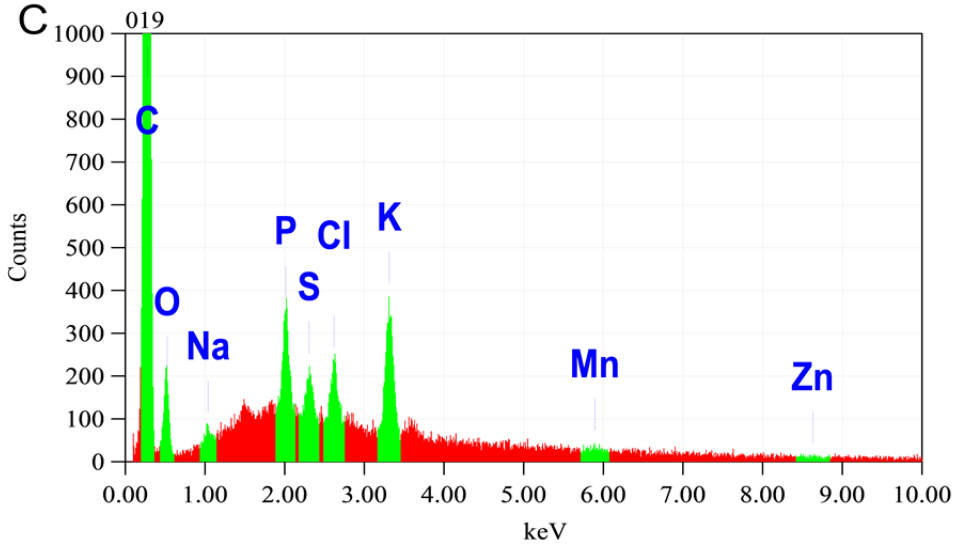
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Figure 5.1 Representative spectra from energy dispersive spectroscopy of aculei taken from *Bactrocera tryoni* (A), *B. oleae* (B) and *Ceratitis capitata* (C) approximately 7 days post-emergence. No elemental peaks corresponding to manganese (Mn) or zinc (Zn) were found in the aculei belonging to the three species of tephritid fruit fly.

5.4 Discussion

While the presence of transition metals has been positively linked to increased cuticle durability, no such metals were detected in the aculei of three tephritids (Schofield *et al*., 2002; 2003; Vincent & Wegst, 2004; Lichtenegger *et al*., 2008). The absence of measureable quantities of transition metals within *B. tryoni* aculei does not necessarily mean that they are less durable than *B. oleae* or *C. capitata* aculei. The mandibular cutting edges of the larval jewel beetle, *Pseudotaenia frenchi* (Coleoptera: Buprestidae) displayed a degree of hardness that compared favourably to some stainless steels, despite the lack of metal-enriched cuticle, and was considerably harder than some adult beetle mandibles enriched with manganese (Cribb *et al*., 2010). Such a result clearly demonstrates that unenriched cuticle is not necessarily softer than enriched cuticle. However, I cannot state that a similar situation has presented itself in this study without quantitative hardness data.

Nanoindentation testing has been used successfully to evaluate the mechanical properties of insect cuticle, including hardness and elastic modulus (Schofield *et al*., 2002; Barbakadze *et al*., 2006; Cribb *et al*., 2010). A geometrically well-defined pyramid is brought into contact with the sample surface and the applied load and displacement (i.e. indentation) curves are used to determine specimen hardness and elastic modulus (Barbakadze *et al*., 2006). I was unable to proceed with nanoindentation testing due to the very difficult challenge of consistiently removing the precise amount of material (5 μm) from the aculeus tip in order to provide a flat surface. Once the relevant technical challenges have been overcome, I recommend that nanoindentation testing should be performed to resolve this question. Chapter 6 will explore an alternative mechanism proposed by some authors as a strategy used by

tephritid fruit flies to avoid aculeus wear; the use of host peel wounds (Papaj *et al*., 1989; Lalonde & Mangel, 1994; Papaj & Alonso-Pimentel, 1997; Diaz-Fleischer & Aluja, 2003c; Nufio & Papaj, 2004).

Chapter 6: Queensland Fruit Fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) Host Utilisation in Response to Host Peel Damage and Conspecific Infestation

6.1 Introduction

The results obtained from observations of *B. tryoni* aculeus wear (Chapter 2) and host preference patterns (Chapters 3 & 4) challenge two assumptions I had developed at the beginning of this project: (i) that *B. tryoni* aculei would show signs of heavy wear after prolonged exposure to oviposition-resistant substrates (Jones & Kim, 1994); and (ii) that ovipositing females would rank soft peeled hosts above hard-peeled hosts, possibly motivated by a desire to avoid cuticle wear (Jones & Kim, 1994; Papaj & Alonso-Pimentel, 1997; Rattanapun *et al*., 2009). Instead, the aculei taken from *B. tryoni* females displayed very limited signs of wear, while host preference patterns indicated that females did not select against hosts based upon peel penetrability. The presence of transition metals has been linked to reduced cuticle wear, but X-ray microanalysis of *B. tryoni* aculei did not reveal measureable quantities of Zn or Mn. The results recorded in this thesis so far appear to contradict our belief about host peel being a limiting trait for fruit fly oviposition. This raises the question of the purpose of fruit wound use reported in fruit fly literature (Pritchard, 1969; Papaj *et al*., 1989; Papaj & Messing, 1996; Papaj & Alonoso-Pimentel, 1997). Consequently, I directed our attention to behavioural mechanisms that might account for the lack of heavy aculeus wear, or the apparent disregard of peel penetrability as a host acceptability cue.

The well-reported tendency of fruit flies to lay their eggs into existing ovipunctures is often referred to as superparasitism, and has been traditionally viewed as a maladaptive behaviour avoided by ovipositing insects due to reductions in offspring fitness (Pritchard, 1969; Papaj *et al*., 1989; Papaj & Messing, 1996; Papaj & Alonso-Pimentel, 1997; Dukas *et al*., 2001; Kano & Harris, 2002; Robacker & Fraser, 2002;

Gonzalez *et al*., 2007). Despite the apparent fitness costs of superparasitism, van Alphen and Visser (1990) proposed that under certain conditions superparasitism can confer significant fitness advantages upon ovipositing adults. Time-limited adults may superparasitise hosts when host density is relatively low, or when female egg load is high (van Alphen, 1990; Dorn & Beckage, 2007; Montoya *et al*., 2013). Consequently, adults enhance their own fitness by increasing reproductive opportunity, although this may come at the cost of reduced offspring fitness (Lalonde & Mangel, 1994; Nufio & Papaj, 2004). The increased metabolic heat produced by a large number of eggs within a single ovipuncture may promote bacterial decay, detoxifying harmful chemical compounds (Diaz-Fleischer & Aluja, 2003a; Rattanapun *et al*., 2009).

The willingness of female tephritids to accept a parasitised host may depend upon volatile semiochemicals emitted from hosts and conspecifics. Chemical cues are used by ovipositing insects during host location, host selection and oviposition site selection (Renwick, 1989; Appolinaire *et al*., 2009; Quilici & Rousse, 2012). The presence of punctures within host peel and the subsequent release of plant specific volatiles may increase the propensity of female insects to oviposit, although some species of tephritids will mark the surface of a host after depositing their eggs with a pheromone that deters other visiting females from using the host (Brevault & Quilici, 2009; Segura *et al*., 2012). Chemical signals may also be associated with eggs and larvae, providing ovipositing females information about the state of the host (Behan & Schoonhoven, 1978; Prokopy *et al*., 1984; Gauthier & Monge, 1999; Prokopy & Papaj, 2001; Liu *et al*., 2011). When presented with unparasitised hosts and hosts containing larvae, gravid Queensland fruit flies avoided depositing eggs into

parasitised hosts (Fitt, 1984; Appolinaire *et al*., 2009; Brevault & Quilici, 2009). This pattern of avoidance persisted even after the larvae had been removed, suggesting the involvement of a chemical component (Appolinaire *et al*., 2009). The lack of discrimination by *B. tryoni* between hosts that bear conspecific eggs and those that are uninfested implies that an inhibitory pheromone is not associated with their eggs (Fitt, 1984). The presence of conspecific eggs within a host may enhance the propensity of tephritid fruit flies to oviposit, as witnessed in the case of the tomato fruit fly *Neoceratitis cyanescens* (Walsh) (Brevault & Quilici, 2009).

The goal of this experiment is to examine the host preference patterns of ovipositing *B. tryoni* females to hosts whose peels have been damaged in different ways, and bear different levels of conspecific infestation. In addition, I also wished to see if the oviposition efficiency (*i.e.* time) of *B. tryoni* females increased or decreased in response to different types of peel damage. A field cage bioassay was performed in order to accomplish these goals, along with a chemical analysis of infested and uninfested hosts.

6.2 Materials & Methods

6.2.1 Overview of Experiment

Host preference patterns were assessed by recording the number of flies upon treated host fruit at hourly intervals. Host handling efficiency was assessed by recording the amount of time taken by ovipositing females to locate a suitable oviposition site and deposit their eggs. Finally, a chemical assay was performed upon host fruit

representing each of the five experimental treatments in order to discern differences in the semiochemical profile of treated hosts.

Five experimental treatments were devised to explore the combined impact of host damage, conspecific infestation and semiochemical olfactory signals upon ovipositing Queensland fruit flies. Each treatment corresponded to five specific combinations of peel damage and conspecific infestation that host fruit were subjected to. The first treatment involved the use of unparasitised hosts whose peels had not been damaged. The second experimental treatment saw me deliberately bruise the peel of the host by dropping the fruit onto a hard surface from a distance of approximately 100 cm. Bruised hosts were used two days after being damaged in order to allow the bruises to become visually distinguishable. For the third, fourth and fifth experimental treatments, I used an entomology dissection pin to artificially puncture the hosts' peel. Five punctures were made each piece of fruit at random locations. The fourth and fifth experimental treatments involved the use of parasitised hosts. In both cases five female flies were allowed to oviposit once in a single fruit, using the artificial ovipunctures. The fourth experimental treatment involved the use of hosts bearing egg infested ovipunctures. Egg infested hosts were used in field cage trials approximately 24 hours after infestation. In contrast, the fifth experimental treatment allowed sufficient time for the development of larvae (4 days) following initial oviposition.

6.2.2 Laboratory Flies

All flies used in field cage observations were obtained from cultures maintained by the Queensland Government Department of Agriculture, Fisheries and Forestry (QDAFF), Boggo Road Ecosciences Precinct, Brisbane. The cultures were up to 34

generations old, refreshed every two generations with wild material and reared on carrot-based medium (Christenson *et al*., 1956). For use in experiments, pupae were received from QDAFF and the emergent adults held under ambient conditions at the Queensland University of Technology. Adults held during experimental trials had *ad libitum* access to sugar, hydrolysed yeast (MP Biomedicals Australasia Pty. Ltd.) and water. Two separate cohorts of flies were ordered, each consisting of approximately 750 individuals. The second population was ordered 5 days after the first. This was done to prevent significant age-related behavioural changes from occurring if only a single population was used. Flies from each population were approximately 9-14 days old when tested. I did not separate flies according to gender in order to minimise the chances of unmated females being used in behavioural trials.

6.2.3 Oviposition Substrates

I used a single type of host as my oviposition substrate during the field cage bioassay. Tomato (*Solanum lycopersicum*) was selected due to its status as a major host of *B. tryoni* (Hancock *et al*., 2000), and was purchased from a local supermarket with the trusses kept intact. Five artificial ovipunctures were made for hosts used in experimental treatments 3-5. A circle 1 cm in diameter was drawn around each ovipuncture in non-toxic marker pen to assist in visual location during behavioural observations. New ovipunctures made by female flies were likewise outlined in pen. Tomatoes used for experimental treatments 3 and 4 were purchased approximately 24 hours prior to use, and were altered according to treatment type on the day of their use. Tomatoes which were used for the fifth experimental treatment (i.e. five artificial ovipunctures wound; infested with larvae) were used 4 days after infestation, allowing

sufficient time for larvae to develop. Tomatoes that displayed obvious signs of damage or were clearly unripe were rejected.

6.2.4 Behavioural Observations

A field cage bioassay was performed to test the host preference patterns and host location/handling times of ovipositing Queensland fruit flies in response to hosts representing five experimental treatments. Two distinct hypotheses were tested during my field cage trials:

1) H0 Host preference patterns of ovipositing Queensland fruit flies did not change in response to different types of host peel damage and conspecific infestation

   H1 Host preference patterns of ovipositing Queensland fruit flies did change in response to different types of host peel damage and conspecific infestation
2) H0 Host finding and handling times of ovipositing Queensland fruit flies did not change in response to different types of host peel damage and conspecific infestation

   H1 Host finding and handling times of ovipositing Queensland fruit flies did change in response to different types of host peel damage and conspecific infestation

In order to test the hypotheses, a 3x3x2.7 metre field cage was established in a residential property located at Sunnybank, a suburb within the Australian city of Brisbane, Queensland. The majority of the trials were conducted daily over a period of fourteen days between April 29 and May 12, 2013. Additional observations for host-handling times were made between May 17 and May 19, 2013. Each behavioural

trial was performed between 0900 and 1600 hours. Ten artificial plants were arranged within the field cage alongside the walls. A single tomato was attached to each of the ten artificial plants by way of their truss, resulting in two representatives for each of the five experimental host treatments. Once the hosts had been prepared, 50 female flies were released into the field cage at 0800 hrs and I commenced observations one hour later. An additional treated host was placed on a small table in the centre of the cage in order to conduct host-handling time observations.

A census of the number of flies upon host fruit attached to artificial plants was taken at hourly intervals along with the measurement of temperature. Flies found upon the surface of host fruit at the end of each hourly census were then removed from the cage and replaced with fresh specimens. Host-handling observations were made continuously throughout the six-hour observation period. Once a female fly alighted upon the surface of a treated host, I placed a 30 cm^3 Perspex observation cage around the host in order to prevent competitive interactions between multiple flies. Using a stopwatch, I recorded the amount of time taken to accomplish three specific oviposition behaviours: (1) the amount of time taken from when a female fly began moving around the surface of the host until it started probing the surface of the host with its aculeus, or its departure; (2) If the fly remained on the host, I then recorded the amount of time taken from the start of aculeus probing until the aculeus had penetrated the surface of the host for five seconds; (3) After five seconds had elapsed, I recorded the amount of time taken until the fly removed its aculeus, completing the oviposition process. A circle was drawn around newly made ovipunctures for hosts belonging to experimental treatments 2-5 in order to easily identify them. When

oviposition occurred in undamaged hosts (i.e. experimental treatment 1), the ovipuncture was sealed with adhesive paper to prevent its reuse by conspecific females and minimise the release of olfactory semiochemicals. At the end of each hourly census, parasitised hosts from treatment 1 used in preference observations were replaced with undamaged specimens. However, I was unable to replace such hosts with fresh specimens owing to time and material constraints.

6.2.5 Semiochemical Analysis

I investigated whether the rejection of larval infested tomatoes by *B. tryoni* might result from changes in odour output. Odours emitted by tomatoes from each of the five treatment groups were collected by headspace analysis of whole fruits (chemical analysis carried out by Paul Cunningham, see Appendix 1). In addition, a headspace analysis was also performed on pierced, four day old tomatoes which were not infested with either *B. tryoni* eggs or larvae.

6.2.6 Data Analysis

Results from hourly censuses and behavioural observations were assessed for meaningful differences according to experimental treatment through one-way analysis of variance (1-way ANOVA) following standard tests for normality (Leven's & Kolmogorov-Smirnov tests) and subsequent Log10 transformations if required. The primary logic for this analysis is that we were most interested to test if different experimental treatments affected host preference patterns and host handling times differentially. The issue of repeated measures by using the same hosts throughout the six hourly censuses was not relevant as I was not interested measuring hourly

differences between treatments. Where data could not be normalised, the nonparametric Kruskal-Wallis test was used as an alternative. Tukey post-hoc pairwise analysis was performed on data sets conforming to the assumptions of a one-way ANOVA, while the Game-Howell post-hoc analysis was used as an alternative for data sets that were normally distributed but did not have equal variance. In the event that a Kruskal-Wallis test was used to test for significant differences between all groups simultaneously, Mann-Whitney U tests were employed to identify significant pairwise differences. All tests were conducted with a confidence interval of 95% and results are presented as the mean ± S.E.

6.3 Results

6.3.1 Host Preferences

I detected a highly significant difference in the number of flies attracted (i.e. visitations) to treated hosts ($H = 75.212$; $P < 0.001$) (Fig. 6.1). Further analysis revealed that the number of visitations to hosts infested with fruit fly eggs or larvae were significantly different compared to uninfested hosts (i.e. undamaged, bruised and artificially pierced hosts). Furthermore, the number of female flies found upon hosts infested with eggs or larvae were significantly different ($M = 5{,}832.500$; $P = 0.006$). Hosts with bruised peel received the greatest mean number of visitors; followed extremely closely by hosts whose peels were artificially punctured and undamaged hosts. Hosts infested with larvae received the least number of visitors in comparison to uninfested hosts and hosts infested with conspecific eggs.

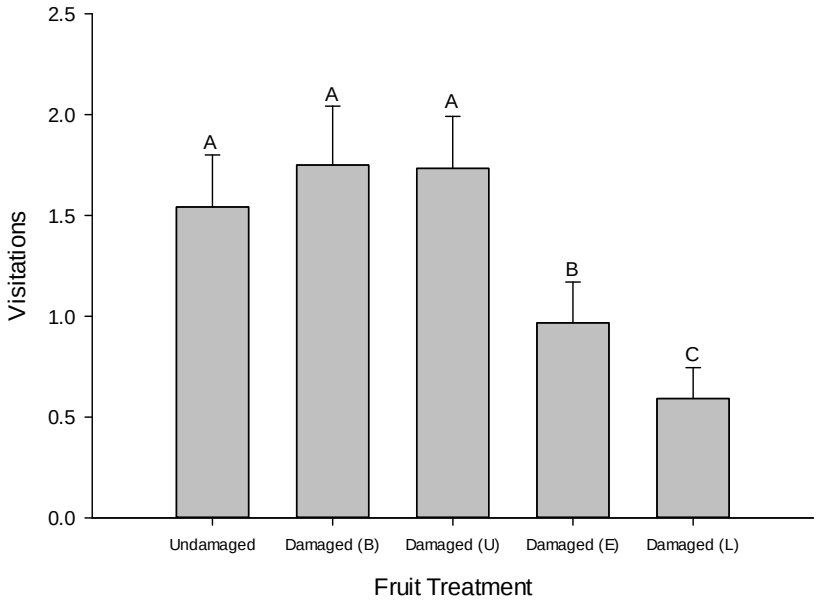


Figure 6.1 Mean (± S.E.) number of visitations to hosts belonging to five experimental treatments: (1) undamaged; (2) damaged hosts with bruised peel; (3) damaged hosts whose peels were artificially pierced; (4) damaged hosts whose peels were pierced and were infested with *B. tryoni* eggs; and (5) damaged hosts whose peels were damaged and contained *B. tryoni* larvae. Columns surmounted with the same letter are not significantly different at $\alpha = 0.05$.

6.3.2 Host Handling Efficiency

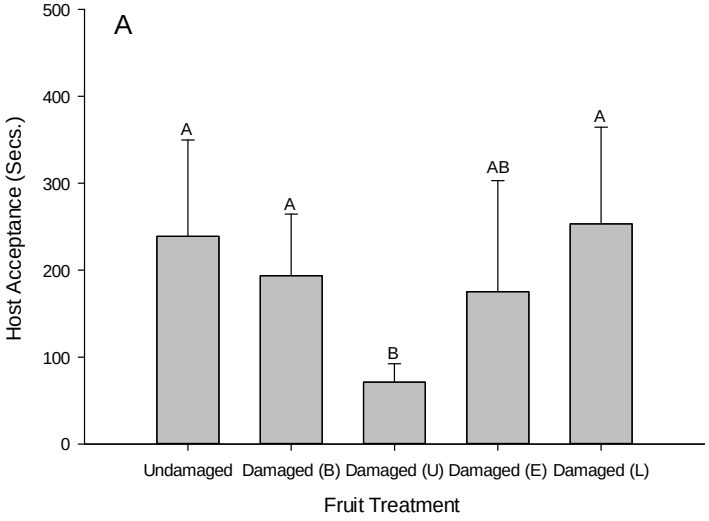
I detected a significant difference between treatments for the total amount of time taken for female flies to complete oviposition ($H = 32.246$; $P < 0.001$) (Fig. 6.2d). Post-hoc analysis revealed that flies using hosts with damaged peel that were either uninfested or infested with eggs (i.e. host treatments 3 and 4) took significantly less time to deposit their eggs compared to flies using intact, bruised and larvae-infested hosts (Figure 6.2). Furthermore, flies exposed to bruised hosts took significantly less time to complete oviposition compared to flies using undamaged hosts (Figure 6.2).

A significant difference between treatments was detected in the amount of time taken from when a fly alighted upon a host until it started probing the surface with its aculeus ($H = 17.081$; $P = 0.002$) (Fig. 6.2a). Post-hoc analysis revealed that flies exposed to uninfested hosts with damaged peel (i.e. host treatment 3) took significantly less time to accomplish this behaviour compared to flies exposed to undamaged, bruised and larvae-infested hosts.

A significant difference between treatments was detected in the amount of time taken from when a fly started probing the surface of the host, until it had pierced the peel with its aculeus ($H = 8.608$; $P = 0.035$) (Fig. 6.2b). Post-hoc analysis revealed that flies exposed to uninfested hosts with damaged peel (i.e. host treatment 3) took significantly less time to accomplish this behaviour compared to undamaged and bruised hosts. I must point out that two observations were excluded from this analysis. The first such observation contained an extremely high value, and was not judged to be representative of the data set as a whole. The second value removed from analysis was the sole observation made for flies exposed to damaged, larvae-infested hosts, which violated the minimum sample size requirement for a Mann-Whitney U test. This was the only time during my observations in which a fly exposed to such a host attempted to pierce its surface.

No significant difference between host treatments was detected for the amount of time taken from when the fly inserted its aculeus into the host until its retraction (Fig. 6.2c).

A greater percentage of flies using hosts with damaged peel (i.e. uninfested and egg infested) successfully completed the entire oviposition process, whereas flies exposed to larval infested hosts did not complete oviposition (Fig. 6.3).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Host Acceptance (Secs.)
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B
AB
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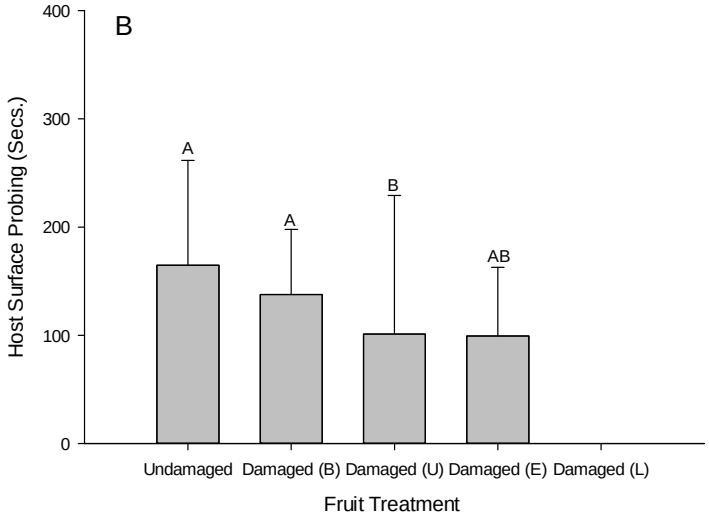
Damaged (B)
Damaged (U)
Damaged (E)
Damaged (L)
Fruit Treatment


B
Host Surface Probing (Secs.)
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Undamaged
Damaged (B)
Damaged (U)
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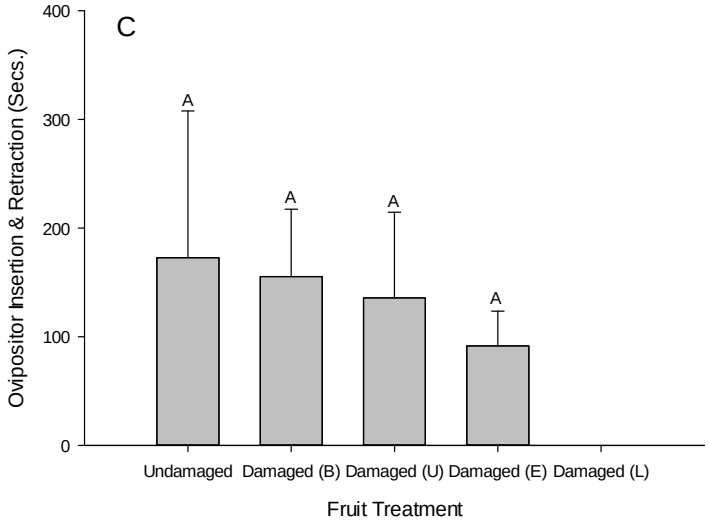
Damaged (L)
Fruit Treatment


C
Ovipositor Insertion & Retraction (Secs.)
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Damaged (B)
Damaged (U)
Damaged (E)
Damaged (L)
Fruit Treatment

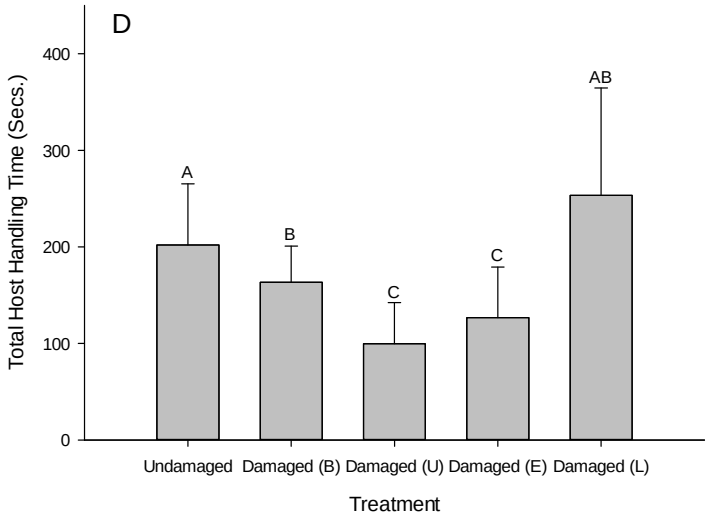


Figure 6.2 Mean (± S.E.) number of seconds recorded from when female *B. tryoni* (A) began walking across the surface of a host until probing the surface via the aculeus; (B) the time taken from the start of surface probing until the insertion of the aculeus; (C) the time taken from when the aculeus was inserted until its removal; and (D) the amount of time taken to complete the entire oviposition process. The five treatments were hosts that were undamaged and uninfested; hosts with bruised peel and were uninfested; hosts whose peels were artificially punctured and were uninfested; hosts whose peels were artificially punctured and were infested with *B. tryoni* eggs; and hosts whose peels were artificially punctured and were infested with *B. tryoni* larvae. Columns surmounted with the same letter are not significantly different at $\alpha = 0.05$.

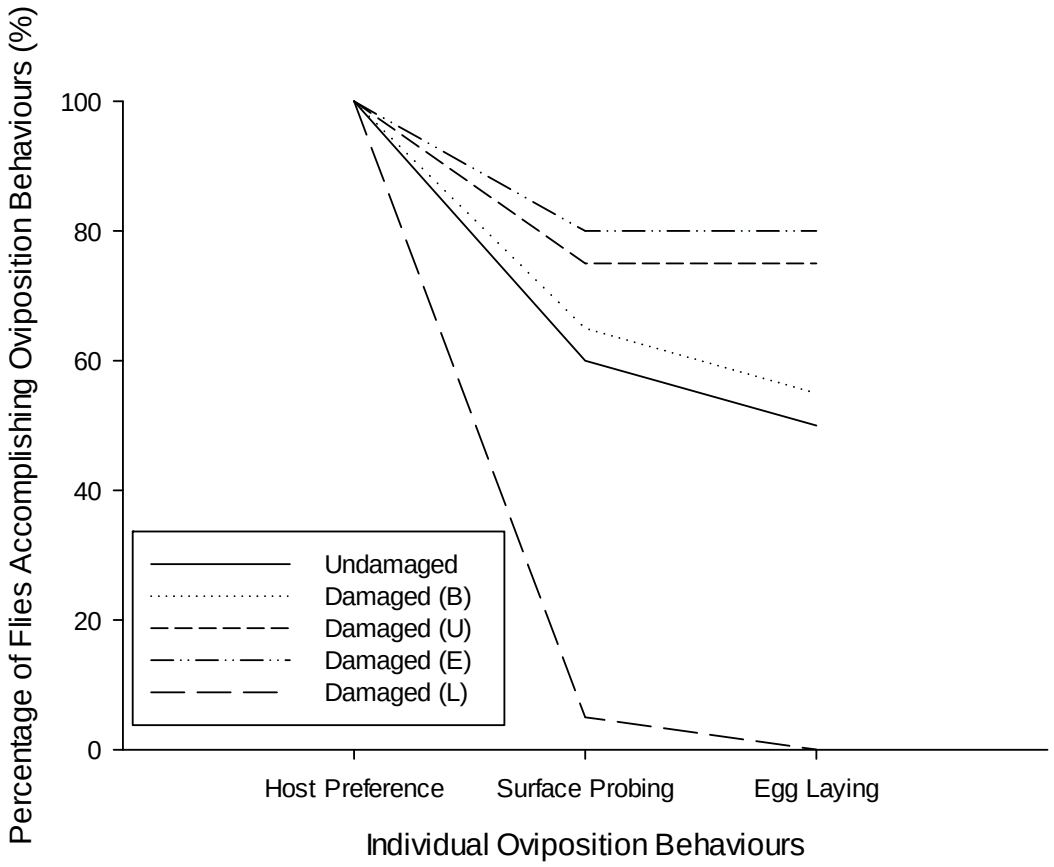


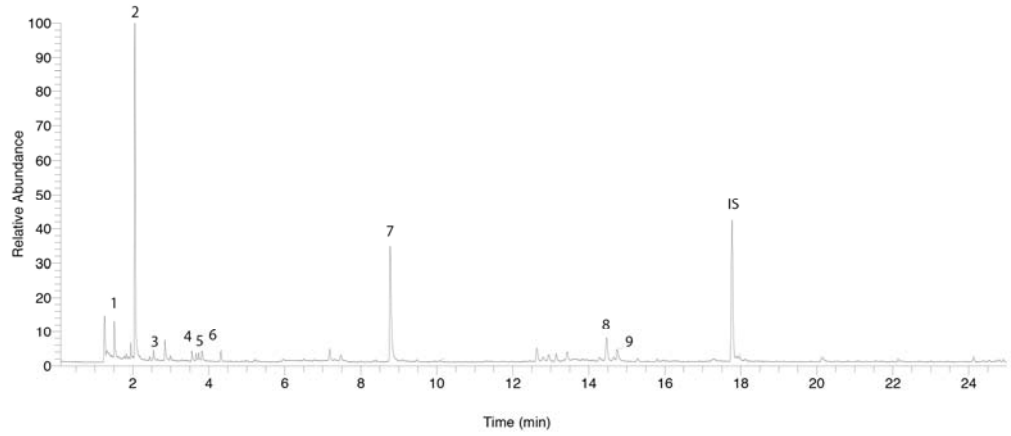
Figure 6.3 Comparison of the proportions of female *B. tryoni* performing three specific oviposition behaviours according to host treatment. Percentages for oviposition behaviours 2 and 3 were calculated by dividing the number of flies that accomplished each behaviour by the total number of observations.

6.3.3 Semiochemical Analysis

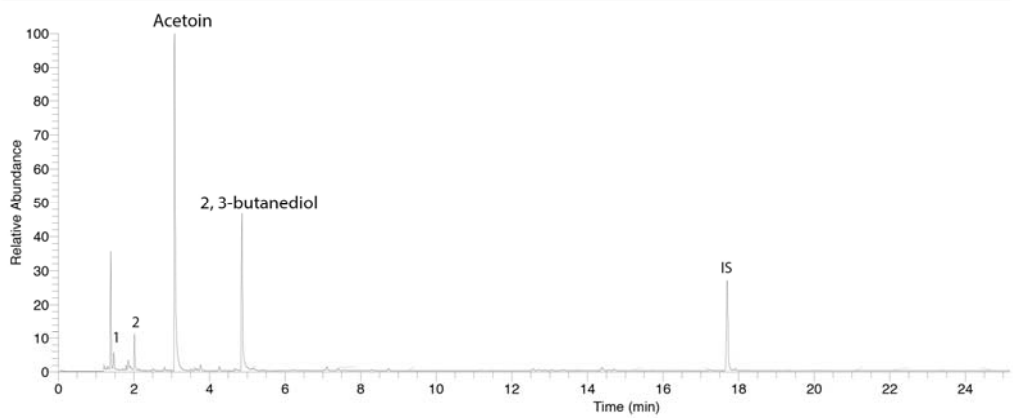
No consistent differences found in the presence or concentration of volatiles in control, bruised, pierced and egg infested hosts. However, all infested fruits (egg and larvae-infested) emitted the volatiles acetoin (3-hydroxy-2-butanone) and 2,3-butanediol (Fig. 6.4b). Acetoin was emitted in relatively large quantities (mean = 8.84 ± 2.37 times the internal standard; N = 5), whereas 2,3-butanediol varied from trace amounts to around four times that of the internal standard (mean = 1.93 ± 0.85 times the internal standard; N=5).

Both these volatiles are produced by bacterial fermentation (Citron, 2012). In this study, larvae-infested tomatoes had been incubated for 4 days after oviposition, and it is possible that mechanical damage to the fruit skin could have led to bacterial infection regardless of whether or not it was performed by the fly's ovipositor.
I therefore carried out an additional trial, analysing headspace odours of tomatoes that had been pierced and then incubated at 28°C for 4 days. Neither acetoin nor 2,3-butanediol were present in odour profiles of these fruits.

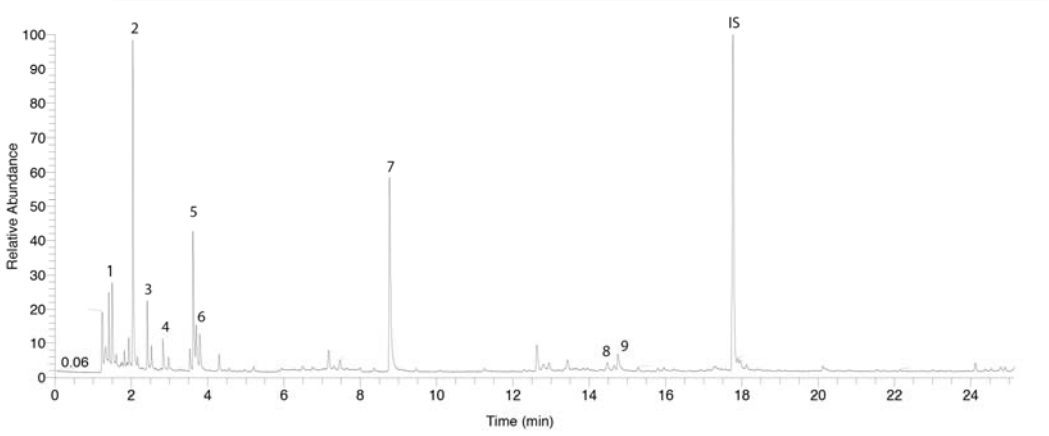
a) Undamaged



b) Infested (4 days)



c) bruised



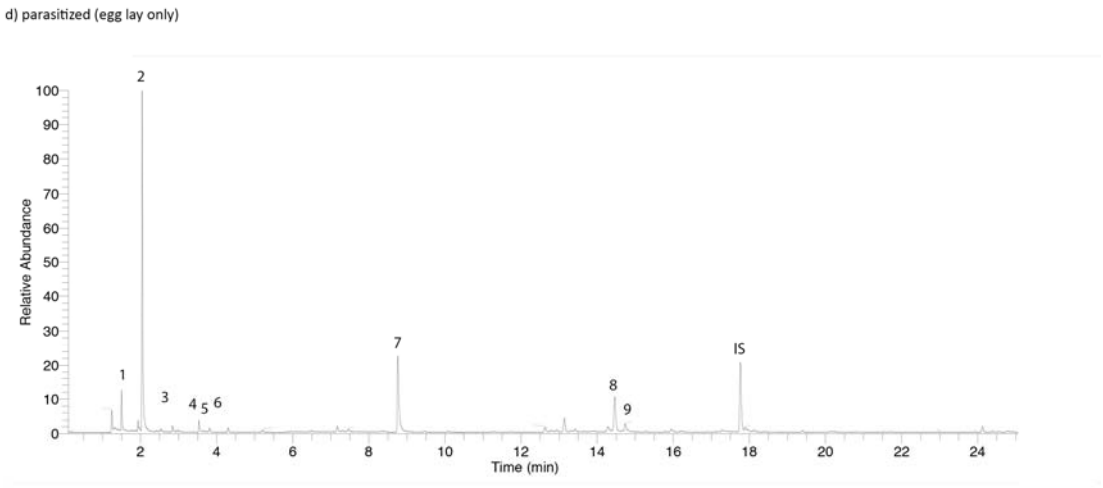


Figure 6.4 Gas chromatograph profiles displaying the relative abundance of volatiles identified within four treatments of tomatoes (a = undamaged; b = larvae-infested; c = bruised; d = egg infested) compared against an added internal standard.

6.4 Discussion

6.4.1 Summary of Results

I found no statistical evidence for a preference by ovipositing flies for damaged versus undamaged fruit. Flies oriented equally to undamaged, bruised and mechanically pierced fruit, although there was a significant decline in use of fruit containing eggs and larvae. The decreased use of these two latter fruit types was possibly due to a chemical deterrent effect and not a physical effect after landing. However, for whole, bruised and mechanically pierced fruit, there were highly significant effects of treatment type on host handling time, with the handling time for pierced fruit shorter than that recorded for whole and bruised fruit. This leads me to infer that the behavioural preference for pierced fruit is a complex ovipositional one, associated with multiple cues but not exclusively driven by peel toughness.

6.4.2 Fitness Benefits Gained from Using Damaged Peel

The use of damaged peel (brought about directly or independently of conspecific oviposition activity) is believed to improve adult fitness by allowing females to save time and energy when exploiting hosts, while decreasing the risks of predation and aculeus damage (Papaj *et al*., 1989; Lalonde & Mangel, 1994; Papaj & Alonso-Pimentel, 1997; Diaz-Fleischer & Aluja, 2003c Nufio & Papaj, 2004). Prior experiments presented in this thesis suggest that peel toughness does act not as a limiting factor for ovipositing *B. tryoni*, nor do their aculei undergo significant wear when exposed to hard-peeled hosts over an extended period of time. However, total host handling time for flies ovipositing onto hosts with intact peel or infested with larvae were typically longer than host handling times for the other host treatments.

Females alighting upon hosts infested with larvae (which they did only rarely), would typically not engage in further oviposition activity (i.e. probing the surface with their ovipositor or depositing eggs). Few flies actually landed on hosts infested with larvae, and there is reason to suspect that volatile chemical emissions from those fruits deterred fruit fly oviposition activity (see further below). In contrast, females using uninfested hosts with damaged peel did show a full range of oviposition behaviours and took significantly less time to start probing the surface of the host after landing, and less time to pierce the peel compared to other treatments. The time taken to deposit eggs after aculeus insertion did not vary significantly between host treatments. I speculate that the shorter time to accept uninfested hosts with damaged peel is related to the release of host-specific volatiles, which is believed to increase the propensity of gravid females to oviposit (Segura *et al*., 2012). Hosts whose peel had been mechanically pierced but contained no larvae (i.e. treatments 3 and 4) saw the

highest rate of ovipuncture reuse (10 and 11 instances, respectively), and this would have led to the shortened time needed for aculeus insertion. Based upon the significantly shorter handling times of flies using hosts with pierced peels, and the lack of evidence that ovipositor wear is a significant issue for this fly, then I believe the evidence is that the reported tendency of *B. tryoni* to reuse ovipunctures is due to female fitness benefits (i.e. reduce vulnerability to predation, optimising host handling time) rather than being a behavioural strategy aimed at reducing aculeus wear or overcoming peel toughness limitations (Papaj & Alonso-Pimentel, 1997). Further testing is required in order to compare the relative rates of aculeus wear in flies exposed to intact and damaged fruit over time, and identify significant differences in host handling patterns between treatments.

6.4.3 Host Preference Patterns in Response to Different Types of Peel Damage

The term 'damaged' is often used generically without taking into account the various ways in which peel might become damaged, which in turn raises the question of how such differences might affect tephritid host preference patterns. Field cage observations strongly indicate that *B. tryoni* females display a strong preference for uninfested hosts, and that hosts infested with eggs were given preference over hosts infested with larvae. Similar results have been reported for *B. tryoni* and other tephritids and chemical emissions produced by hosts and conspecifics have been linked to host avoidance behaviour (Fitt, 1984; Appolinaire *et al*., 2009; Brevault & Quilici, 2009). Although more flies were found on the surfaces of uninfested hosts whose peels were mechanically punctured or bruised than on undamaged hosts, statistical analysis revealed this difference to be insignificant, suggesting that females

can perceive the presence of eggs despite the presumed lack of semiochemical signals (Brevault & Quilici, 2009).

6.4.4 Semiochemical Odours and Tephritid Oviposition Behaviour

It has been demonstrated that phytophagous insects use semiochemicals produced by conspecifics and other organisms to identify nutrient resources, competitors, predators, potential mates and habitat suitability (Renwick, 1989; Appolinaire *et al*., 2009; Quilici & Rousse, 2012; Davis *et al*., 2013). Host marking pheromones (HMPs) produced by conspecific adults, larvae and eggs have been studied within the context of tephritid oviposition behaviour, and there is evidence suggesting that they may inhibit subsequent females from depositing their eggs (Behan & Schoonhoven, 1978; Roitberg *et al*., 1984; Gauthier & Monge, 1999; Prokopy & Papaj, 2001; Brevault & Quilici, 2009; Liu *et al*., 2011). Alternatively, the release of host-produced semiochemicals through wounded peel may also increase the propensity of gravid females to oviposit (Segura *et al*., 2012). Less well-studied is the role played by semiochemical emissions produced by microbial organisms including bacteria and fungi (Davis *et al*., 2013). Although there is very little information regarding the impact of such emissions upon tephritid fruit flies, Lauzon *et al*. (1998) demonstrated that semiochemical volatiles produced by the bacteria *Enterobacter agglomerans* increased oviposition rates of *R. pomonella*. The headspace analysis of four day old hosts containing larvae indicated large quantities of the bacterial metabolite acetoin. Acetoin is a by-product of alcoholic fermentation linked to the activity of certain rhizobacterial *Bacillis* strains within overripe fruit (Schulz & Dickschat, 2007; Ruebenbauer, 2009; Becher *et al*., 2012). Acetoin serves as a component in a blend of five compounds which acts as an attractant to the fruit fly *Drosophilia melanogaster*,

which lays its eggs into rotten fruit (Ruebenbauer, 2009; Becher *et al*., 2012). In contrast, *B. tryoni* did not use larvae-infested hosts, which I recorded as producing high levels of acetoin, nor does the fly use rotting hosts (Prokopy *et al*., 1989). Thus, while the rejection of infested hosts by *B. tryoni* is unlikely to be attributed solely to the presence of acetoin within the host, there is circumstantial evidence that acetoin may be linked to *B. tryoni*'s rejection of infested hosts. Additional study would be needed to confirm the role of acetoin in host rejection decisions by *B. tryoni*. Finally, the likely presence of decay bacteria in overripe tomatoes may also deter use of such fruit by *B. tryoni* – but trials to demonstrate such an effect would be needed.

Chapter 7: Final Discussion

7.1 General Discussion

In this thesis I investigated the effect of host peel toughness on host range in the polyphagous Queensland fruit fly. Specifically, I inquired whether *B. tryoni* would accept or reject hosts based upon the penetrability of host fruit exocarp and the presence of peel punctures. In a related line of inquiry, I also examined whether the aculei of *B. tryoni* would experience different levels of abrasive wear after prolonged exposure to soft and hard-peeled hosts. If significant wear did occur, would I observe a narrowing of host range as opposed to flies with unworn aculei? However, if wear did not occur, what might be the mechanisms responsible? I started this thesis with two key assumptions: (i) that the aculei of fruit flies exposed to hosts with hard peel over an extended period would become heavily worn; and (ii) that ovipositing *B. tryoni* females would display a preference for soft peeled hosts

Caged populations of *B. tryoni* were exposed to a variety of natural hosts and agar fruit mimics for seven weeks (Chapter 2). Natural hosts represented a spectrum of different peel properties (i.e. thickness, elasticity and hardness), while agar fruit mimic treatments differed in terms of density (i.e. low, medium and high). I predicted that the aculei of flies exposed to hard peeled hosts / high-density mimics would be heavily worn. In addition, I also predicted that aculei taken from flies at the end of the seven weeks would be more heavily worn than aculei sampled at the beginning of the study. These predictions were based on the results of Jones and Kim (1994), who noted heavily worn aculei from field-caught specimens of tephritid fruit flies. However, my results showed that aculeus wear in *B. tryoni* as a product of oviposition substrate or time was very limited.

Laboratory-based behavioural observations (Chapters 3 and 4) suggested that increased peel toughness or peel surface properties (i.e. surface texture and wax) did not act as deterrents to ovipositing *B. tryoni*. In particular, during no-choice observations in Chapter 4, I noted that fruit flies ranked passionfruit the highest in terms of visitations despite their highly resistant peel: larval survival trials indicated that passionfruit was the highest quality host amongst my treatments. Papaya, which was classified as a poor host for offspring, received the least number of visitations despite having an easily penetrable peel. These results led me to select the preference-performance hypothesis (sensu Scheirs *et al*., 2004; Heisswolf *et al*., 2005) as a convincing rationale for the observed host utilisation pattern. Although there are numerous examples of polyphagous insects selecting unsuitable hosts in order to increase individual parental fitness (Mayhew, 2001; Scheirs, 2002 & De Bruyn; Uesugi, 2009), high quality passionfruit being visited more often than low quality papaya directly matches predictions which might be made about the parental female choosing oviposition substrates based on maximising offspring performance.

The unworn aculei observed in Chapter 2 coupled with the selection of hard-peeled hosts in behavioural experiments encouraged me to explore mechanisms which might explain the low levels of wear observed. There is mounting evidence that cuticle enriched with transition metals such as zinc will have increased durability, and that concentrations of such metals will occur in areas subjected to frequent contact with external objects (Fontaine *et al*., 1991; Quick *et al*., 1998; Schofield *et al*., 2003; Vincent & Wegst, 2004; Broomell *et al*., 2008). I therefore predicted that the aculeus tip of *B. tryoni* would be enriched with zinc or similar metals, whereas those of *C.*

capitata and *B. oleae* (for which wear has been demonstrated) would not. However, X-ray microanalysis indicated that *B. tryoni* aculei tips were not enriched with transition metals, nor were transition metals detected in the aculei taken from *B. oleae* and *C. capitata*. The lack of transition metals within the cuticle of fruit fly species used in this thesis may reflect the use of an artificial diet for developing larvae. Transition metals are thought to be acquired from the tissues of the larval host and incorporated within the cuticle multiple times as the larvae passes from one instar to the next (Hunter *et al*., 1987; Quicke *et al*., 1998). The cultures from which fruit fly specimens were taken from in Chapter 5 were provided with a standard dried-carrot-based diet, which may not have exposed developing flies to the transition metals they could expect from natural fruit hosts. However, *B. tryoni* females used in Chapter 2 were likewise given a carrot-based diet, and the limited amount of aculeus wear observed in Chapter 2 when compared to the aculei observed by Jones and Kim (1994) suggests that cuticle wear resistance in *B. tryoni* is not dependent upon the presence of transition metals.

The second mechanism considered to minimise fruit fly aculei wear is the use of damaged or soft sections of host peel. This behaviour is typically discussed within the context of superparasitism, a behaviour in which flies will reuse the ovipunctures made by conspecific females (Papaj *et al*., 1989; Lalonde & Mangel, 1994; Papaj & Alonso-Pimentel, 1997; Diaz-Fleischer & Aluja, 2003c Nufio & Papaj, 2004). In addition to reducing wear, flies taking advantage of damaged peel are also thought to spend significantly less time in the act of oviposition, leaving them less vulnerable to predation. I investigated the host-use patterns of *B. tryoni* to tomatoes with intact and damaged peel, and for the latter with or without conspecific infestation of fruit.

Although flies did not demonstrate a significant preference for hosts with punctured peel, such hosts were used more efficiently (i.e. quickly). Flies only rarely alighted on fruit with pierced peel which were infested with larvae, and headspace analysis of odours from those fruit revealed significant quantities of the bacterial metabolite acetoin, which is often detected in fermenting fruit.

Increased host-peel toughness is believed to limit the ability of fruit flies to successfully access hosts, and that fruit flies choose soft or damaged fruit to help overcome peel penetrability problems and to minimise aculeus wear (Pritchard, 1969; Bateman, 1972; Diaz-Fleischer & Aluja, 2003b; Rouquette & Davis, 2003; Aluja *et al*, 2004; Balagawi *et al*., 2005; Okolle & Ntonifor, 2005; Sharma & Amritphale, 2008; Rattanapun *et al*., 2009, 2010). In my study system, the polyphagous fruit fly *B. tryoni*, I conclude that host-use decisions are not made on peel attributes, and that everything except fruit of extreme peel toughness can be utilised as a host with little resultant ovipositor wear. Rather, the preferential use of fruit with damaged peel has significant time saving benefits to female flies, which may (although this was not assessed) have flow-on benefits in terms of minimising predator induced mortality or optimising oviposition behaviour.

7.2 Oviposition, Host Range and Polyphagy

The complex host utilisation patterns of phytophagous insects are commonly reduced to long lists of plants from which the insect has, even if only once, been recorded (e.g. Allwood *et al*., 1999; Hancock *et al*., 2000). Such host lists have received criticism for not taking into account the ecological circumstances ovipositing insects face when

selecting hosts under natural conditions (Balciunas *et al*., 1996; Van Klinken & Heard, 2000). The long list of host names assigned to fruit flies such as *B. tryoni* or *C. capitata* fosters the impression that polyphags treat all hosts with equal consideration, although comparisons between host lists and host preference tests have demonstrated that polyphagous fruit flies do not use hosts indiscriminately (Fitt, 1984; Clarke *et al*., 2005; Rwomushana *et al*., 2008). Finally, even the conventional definition of 'polyphagy' is inadequate. A major review of fruit fly host-use proposed that: "*...in terms of host cues to which they respond when searching for and accepting oviposition sites [polyphagous tephritids] are chemical, visual, and tactile generalists*" (Diaz-Fleischer *et al*., 2001). The problem with this argument is that it is circular. According to Diaz-Fleischer *et al*. (2001) polyphagous insects occur because they are "generalists", but by definition polyphagous insects are generalists, and so simply saying that they have a generalist response offers no new insights (Walter, 2003).

Studies on the highly polyphagous tephritid *B. dorsalis* have provided evidence that ovipositing females discriminate between hosts (Clarke *et al*., 2001; 2005), with improved offspring performance proposed as the evolutionary driver (Rattanapun *et al*., 2010). This interpretation of *B. dorsalis* host preference patterns has been found in other systems, such as the polyphagous comma butterfly (*Polygonia c-album* Linnaeus), in which positive host selection was linked to increased larval development rate (Nylin, 1988). However, positive preference/performance relationships have been found not to explain host-use patterns in some herbivores, including other studies of *B. tryoni* (Balagawi *et al*., 2013), and illustrate the complexity of interpreting host-use studies in the light of over-arching theories.

Finally, differences between broad 'potential' host ranges which are often witnessed in laboratory studies and the narrower 'actual' host ranges observed in field studies must also be recognised (Balciunas *et al*., 1996; Morehead & Feener Jr, 2000; Van Klinken & Heard, 2000). That the broad 'potential' host range of phytophagous insects is invariably larger than their actual host range has been attributed to the artificial conditions insects are subjected to in laboratory studies (Morehead & Feener Jr, 2000) and demonstrate that host range classifications, often dependent upon host preference/association patterns, should not be accepted uncritically. I believe that examining host range purely from a pattern-based perspective (i.e. preference/performance, monophagy versus polyphagy) will shed few insights, and that a process-based approach to insect host-use may be more useful. My work on understanding the behavioural basis of oviposition decisions in *B. tryoni*, and the physical aspects of host-use and ovipositor wear, is part of that mechanistic approach.

Fruit peel resistance to oviposition is considered an important factor in tephritid oviposition behaviour (Diaz-Fleischer & Aluja, 2003b; Rouquette & Davis, 2003; Aluja *et al*., 2004; Balagawi *et al*., 2005). More specifically, a highly resistant host peel is thought to restrict fruit fly access to the host, and in doing so reduce the insect's host range (Balagawi *et al*., 2005; Dhillon *et al*., 2005; Muthuthantri & Clarke, 2012). Additionally, a loss in ovipositor effectiveness is believed to contribute to a reduction in host range (Jones & Kim, 1994). The loss of efficiency in flies that have undergone cuticular wear is thought to have lead to the evolutionary adoption of strategies intended to reduce or avoid wear, such as using damaged areas of host peel. Prior behavioural studies have shown a preference by tephritid fruit flies towards hosts with easily penetrated peel (Balagawi *et al*., 2005; Sharma &

Amritphale, 2008), although there is also evidence suggesting that fruit flies may deposit larger egg clutches in fruit with hard peel (Diaz-Fleischer & Aluja, 2003b), a trait seen as an indirect marker of high quality hosts (Greany *et al*., 1985; Messina & Jones, 1990). While the impact of host peel properties upon tephritid oviposition behaviour (i.e. clutch size, use of fruit wounds) has been investigated in previous studies, questions relating to peel properties and host range have not been addressed.

The results obtained in this thesis suggest that *B. tryoni* does not use hosts indiscriminately. In my study I have observed that increased peel penetration resistance and elasticity do not deter ovipositing *B. tryoni* for hosts of high offspring quality. This preference pattern matches the prediction of the preference-performance hypothesis of herbivore host-use (Scheirs *et al*., 2004; Heisswolf *et al*., 2005). Nevertheless, field cage studies in Chapter 6 demonstrate that additional factors underlie *B. tryoni* host selection, including the presence of damaged peel and semiochemical emissions. Although the use of damaged peel is often interpreted as a strategy to avoid aculeus wear, I noted that flies ovipositing into damaged hosts took significantly less time to oviposit than those using intact or bruised hosts. I believe that the detailed investigations into the mechanistic interactions between *B. tryoni* and host fruit have identified key processes driving host association patterns, including an avoidance of low larval quality hosts, avoidance of maggot infested hosts through chemical signals, and minimisation of host handling time. Further studies on *B. tryoni* host-use should look to these different processes, both singly and in combination, to better resolve research conflicts (e.g. my results in Chapter 4 versus those reported in Balagawi *et al*., 2013).

7.3 Gaps in Knowledge

7.3.1 Increased Age and Loss of Vigour in Tephritid Fruit Flies

Although recent studies into insect oviposition have concentrated on physiological factors (e.g. egg load), the importance of age and physical wear is still open to question (Tammaru & Javois, 2005; Schofield *et al*., 2011). Carey *et al*. (1998) theorised that because of the stress and high energy expenditure associated with oviposition, fruit fly females that are reproductively active at young ages should become weaker as their age increases, which should be manifested as decreased fecundity or increased mortality. However, in the same paper, Carey *et al*. found no correlation between the number of eggs laid by young *C. capitata* and subsequent reproduction and life span, although the authors cautioned that these results may be attributable to the controlled conditions to which female flies were exposed. Similarly, Jang & Light (1991) recorded a peak response in female *B. dorsalis* to ripe papaya 8-10 days after emergence followed by a decline, which was subsequently followed by another response peak 15-16 days post-emergence. These examples indicate that increased age does not necessarily result in reduced oviposition activity and, at least for my system, the mechanisms of oviposition also do not suggest an age effect.

My analysis of aculei removed from 49 day old *B. tryoni* showed no statistically significant signs of wear compared to newly emerged flies. While increased aculeus wear and (an inferred) resultant inability to access hosts has been associated with aging in tephritids (Jones & Kim, 1994), I think it is highly unlikely that the limited wear I observed in *B. tryoni* females will prevent these flies from successfully using

hosts. However, no studies (including my own) have been performed that specifically examine the effects of age upon host range and oviposition behaviour in *B. tryoni*. While a female *B. tryoni* might retain a sharp ovipositor as she ages, a loss in general vigour may restrict host range.

7.3.2 Comparative Analysis of Ovipositor Anatomy & Morphology

Recent investigations into species of *Bactrocera* and *Ceratitis* have revealed noticeable differences in ovipositor musculature which have been speculated to be related to a fly's ability to effectively use host fruit (Ovtshinnikova, 2012). White (2001) compared aculeus morphology between species of tephritid fruit flies belonging to the genera *Dacus* and *Bactrocera*, and proposed that the fusion of ovipositor tergites seen among *Dacus* spp. might confer an advantage for oviposition into thick peeled hosts (White, 2001). Furthermore, differences in ovipositor morphology (i.e. apex sharpness, length, width) have been associated with the ability of phytophagous insects (even within populations of the same species) to effectively use hosts (Jones *et al*., 1993; Balagawi *et al*., 2005; Sayar *et al*., 2009). Given this background, I would expect that the structure of the ovipositors of *B. tryoni* and *C. capitata* would be very similar as both species have extremely broad host ranges and their aculeus morphology is generally similar (White, 2001). However, there is a clear difference between the species in terms of aculei resistance to abrasive wear (i.e. results of Chapter 2 vs. Jones & Kim, 1994), and I considered this unexpected. The wear resistant nature of *B. tryoni* aculei is not, however, due to the inclusion of transition metals (which I predicted would be present in *B. tryoni* and absent in *C. capitata*) and indicates that wear resistance is a complex phenomenon for which it is unlikely that a single factor is responsible. I believe that further comparisons of

ovipositor anatomy and elemental composition should be performed in order to identify host range-specific features, or lack thereof, in *B. tryoni* and other tephritid species.

7.3.3 Semiochemical Analysis

Phytophagous insects use olfactory semiochemicals emitted from hosts and conspecifics to locate and select suitable hosts (Renwick, 1989; Appolinaire *et al*., 2009; Quilici & Rousse, 2012). Further, the release of specific plant semiochemicals through damaged peel is believed to positively stimulate fruit fly oviposition behaviour (Segura *et al*., 2012). Alternatively, ovipositing insects can be deterred through the presence of host marking pheromones produced by conspecific adults, larvae and eggs (Prokopy *et al*., 1984b; Liu *et al*., 2011). However, the effect of semiochemical emissions produced by microbial organisms upon tephritid oviposition behaviour has not been studied in depth. Headspace analysis of tomato treatment groups in Chapter 6 revealed that four day old tomatoes infested with larvae contained elevated amounts of the bacterially produced chemical, acetoin. I concluded that acetoin, produced in fermenting fruit by bacteria, may exist as part of a complex volatile blend that deters *B. tryoni* from depositing eggs into rotten hosts. Further effort should be made in order to identify the specific chemical components and the roles they play in deterring *B. tryoni* oviposition, and whether microbial organisms associated with fermentation are transferred directly to the host by the adult, or arrive afterwards.

7.4 Final Conclusion

By adopting a mechanistic-based approach and examining the behaviours of ovipositing *B. tryoni* females in response to peel properties, I believe this study has enhanced our understanding of host utilisation patterns in this fly, and frugivorous tephritids more generally. Although I concluded that increased peel toughness did not deter ovipositing females from using hosts such as passionfruit or rockmelon, it is important to add the caveat that other researchers have found the opposite for *B. tryoni* (Balagawi *et al*., 2005; Muthuthantri & Clarke, 2012). It should be noted, however, that these other studies were not explicitly designed to test the role of peel properties on host preference, but simply recorded peel toughness as one of several host variables correlated with host preference and performance. They did not, as I did, differentiate the different steps of the host-use process and so identify where peel toughness became a physically limiting step, i.e. they did not separate the difference between adult preference versus physical ability to penetrate peel: as I demonstrated for passionfruit these can be quite different things. Nevertheless, as with differences between the predictions of different theoretical host range models (Balagawi *et al*., 2013), the discrepancies reported between the host utilization patterns for *B. tryoni* in response to peel toughness reinforces the point that we are dealing with a complex suite of behaviours which are dependent upon the interaction of numerous physiological and environmental factors. Each additional study such as mine, which focuses on explaining mechanistic patterns of host-use rather than testing theoretical predictions of host-use, helps unravel this complexity.

To summarise, the conclusions of this thesis are:

- Limited wear was observed for *B. tryoni* aculei in response to oviposition substrate and time.
- *B. tryoni* females did not demonstrate a consistent preference for soft peeled hosts, or for rough peeled and unwaxed artificial hosts, which existing literature predicted should have been preferred.
- *B. tryoni* females displayed a preference for hard-peeled hosts which enhanced offspring survival, as opposed to soft peeled hosts which decreased offspring survival.
- Aculei taken from *B. tryoni, B. oleae* and *C. capitata* specimens did not contain noticeable quantities of hardness-enhancing transition metals.
- Significant time savings were observed in ovipositing *B. tryoni* females using hosts whose peels had been pierced.
- I conclude that the use of wounds or soft spots is related to time saving, not avoiding aculei wear or the need to overcome peel penetration resistance.

Chapter 8: References

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